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CONTENTS—INHOUD

A MODIFIED BROMINE VAPOUR METHOD FOR THE DETERMINATION OF IODINE VALUES

by

M. ATMORE and F. HAWKE

OPSOMMING

'n Makrometode is ontwikkel vir die bepaling van joodgetalle deur die direkte inwerking van broomdamp by gewone temperatuur. Die oormaat broom word onder verminderde druk en deur middel van 'n stroom warm stikstof of lug verwyder.

Die metode is toepaslik in die geval van hoog-onversadigde olies wat vetsure met drie gekonjugierde dubbelbindings bevat maar nie in die geval van gehidroksileerde verbindinge nie, omdat substitusie in laasgenoemde geval plaasvind.

SUMMARY

A macro-method has been developed for the determination of iodine values by the direct action of bromine vapour at room temperature. Excess bromine is removed under reduced pressure and by means of a stream of warm nitrogen or air.

The method is suitable for highly unsaturated oils containing conjugated tri-ethenoid fatty acids, but cannot be used for hydroxylated compounds owing to the occurrence of substitution reactions.

While the iodine value is one of the most widely used analytical determinations in the field of fat chemistry, methods in use today are not altogether satisfactory, expecially for the analysis of fats containing conjugated unsaturation.

Although suitable for most purposes, the methods of Wijs[1] and Hanus[2] both give low and unreliable results with fats containing esters of elaeostearic and licanic acids. A modification of the latter method, proposed by von Mikusch and Frazier,[3] was found to depend too greatly on rigid adherence to closely controlled conditions and, while giving better results than the earlier methods, was consequently of doubtful accuracy.

After a survey of the literature it was decided to investigate more fully the bromine vapour method of Hehner[4] together with modifications by Becker,[5] Toms,[6] Rossman[7] and Boeseken and Pols.[8]

In order to ensure as wide an applicability as possible, determinations were carried out on various samples of tung oil, a number of less highly unsaturated oils and pure methyl esters.

The methods proposed by Hehner[4] and subsequent workers[5, 6, 7, 8] consist in the exposure to bromine vapour of a weighed film of oil on a glass plate. Excess bromine is then removed and the brominated oil reweighed.

With the exception of Sabalitschka and Dieterich,[9] who used 0·1 to 0·4 g. of oil on a large glass plate, published modifications of this method are carried out on a microscope slide using sample weights ranging from 1 to 25 milligrams. These latter methods are unsuitable for routine purposes, since they require the use of a microbalance, while Sabalitschka and Dieterich[9] observed substitution during removal of the excess bromine by prolonged heating.

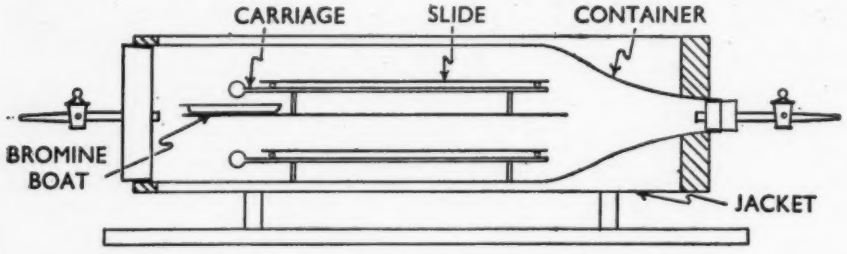
The following investigation was carried out in order to develop an accurate method for the determination of the total unsaturation of highly unsaturated compounds while maintaining as wide an applicability as possible.

Sample weight

In order to achieve a precision of $\pm 0{\cdot}3$ per cent while retaining the use of an ordinary analytical balance, a sample weight of 80 to 120 milligrams was selected. This necessi-

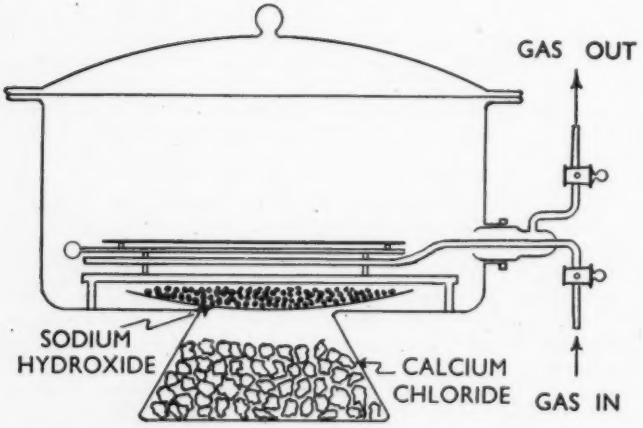
tated an area of about 100 sq. cm. if the oil film was to be sufficiently thin to ensure complete removal of excess bromine.

A glass plate of adequate size proved very cumbersome to weigh and required a large brominating chamber. By mounting the plate horizontally on a small glass carriage (Fig. 1) both sides could be coated with films of oil and the size reduced to 10 by 5 cm. Some difficulty was experienced in obtaining oil films of even thickness but this was overcome by using glass plates coarsely ground on both sides.

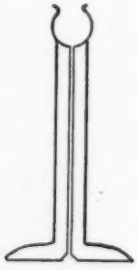


BROMINATION APPARATUS

Fig. 1.



APPARATUS FOR REMOVAL OF EXCESS BROMINE.



SPREADER (SECTIONAL).

Fig. 2.

Application of oil to the plate

Toms[6] applied the film of oil to the plate by spreading a drop with his finger. To eliminate the possibility of contamination from this source, a glass spreader was used (Fig. 2). This was made from 1 mm. bore capillary tubing. A cup was formed by removing the top of a small bulb blown on one end of the tube while the spreading disc was formed by thickening the glass appreciably and cutting and polishing with jeweller's rouge. Two or three drops of oil were introduced into the cup at the top of the spreader and the rate of delivery of oil to the glass plate controlled by means of a finger over the opening to the cup. In this way films 0·001 to 0·0015 cm. thick could readily be obtained without contamination.

Removal of excess bromine

Since heating as a means of removal of bromine had proved unsatisfactory on a macro scale,[9] the use of reduced pressure in the presence of sodium hydroxide was investigated. Although this treatment alone resulted in a satisfactory removal of the excess bromine, the time required was too great in most cases. To accelerate the removal of bromine the low pressure treatment was supplemented by warming the plates in a current of nitrogen or air at 80°C.; followed by further vacuum treatment while still warm. It was found that three such treatments were sufficient to bring the weight of brominated oil to a constant value. The total time required for the removal of excess bromine was 30 minutes, while the films were exposed to warm gas for only a third of this time.

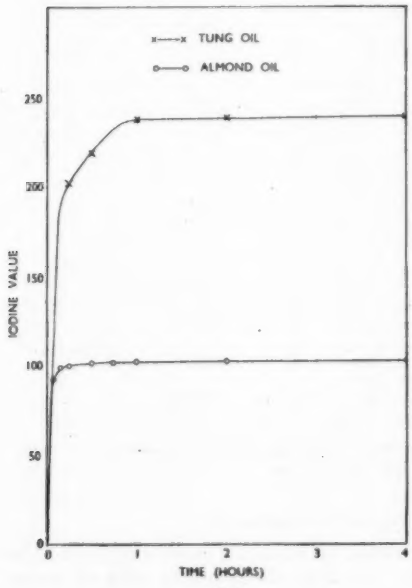


FIG 3

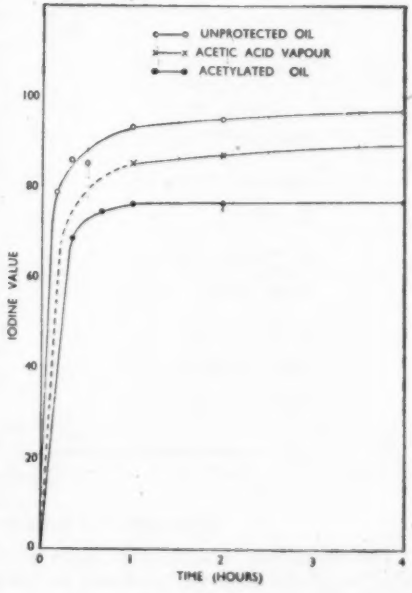


FIG 4

Possibility of substitution

In attempts to evaluate substitution reactions, tests were carried out on purified palmitic acid; values of 0·6; 0·4 were obtained (Wijs 0·4; 0·2) indicating that with saturated fatty acids substitution reactions were not appreciable. Due to the possibility of substitution being catalysed by the presence of double bonds the method of Brocklesby and Harding[10] was used. No hydrogen bromide was detected, indicating the absence of substitution reactions by the action of bromine vapour on unsaturated fatty acids and their esters under the conditions used.

Time of bromination

In order to study the effect of time of exposure to bromine vapour, experiments were carried out on tung, almond and castor oils. The results of these determinations are shown graphically (Figs. 3 and 4). With tung and almond oils constant values were obtained after one hour, but in the case of castor oil anomalies resulted. The iodine value continued to increase and rose considerably above the value obtained by the Wijs and Hanus methods. Further experiments using a number of drying, semi-drying and non-drying oils as well as methyl esters of high purity gave very satisfactory results.

TABLE I

Material	*Iodine Value (Bromine vapour)*	*Iodine value (Wijs)*[1]
Linseed oil ... ... (1)	176·2, 175·9 Mean: 176·1	175·8
(2)	185·6, 184·8 Mean: 185·2	185·8
Tung oil ... ... (1)	234·5, 233·7 Mean: 234·1	—
(2)	197·0, 198·2 Mean: 197·6	154·0 (190·1*)
Maize oil.	111·7, 111·3 Mean: 111·5	110·9
Sesame oil	112·8, 113·2 Mean: 113·0	113·0
Palm oil	57·8, 57·6 Mean: 57·7	58·2†
Elaeostearic acid (crude, m.p. 69°C.)	264·6, 265·0 Mean: 264·8	—
Methyl oleate	85·9, 85·6 Mean: 85·8	86·4
Methyl linoleate	171·0, 171·3 Mean: 171·2	172·1
Methyl linolenate	259·5, 259·4 Mean: 259·5	260·0
Ricinoleic acid	96·6, 98·6 Mean: 97·6	85·26

*Woburn.[3] †Hanus.[3]

The action of bromine vapour on castor oil

The anomalous behaviour of castor oil was noted by Rossman[7], who claims that the addition of bromine vapour could be used to determine the hydroxyl value of castor oil as well as the iodine value.

In order to confirm that the behaviour of castor oil was due to the presence of free hydroxyl groups, determinations were carried out on a sample of acetylated castor oil when no anomalies were observed (Fig. 4).

In an attempt to make the method applicable to castor oil, samples were brominated in an atmosphere saturated with acetic acid vapour. Although an improvement was effected (Fig. 4), the results did not justify further investigation along similar lines.

The effect of light on the iodine value

Samples of almond oil were brominated in diffused daylight. Values of 100·4 and 100·7 were obtained, compared with 100·3 and 100·5 when light was excluded. Although the increase noted was very slight it was felt desirable to eliminate the possibility of a photocatalytic effect promoting substitution and the brominating chamber was accordingly enclosed in a sheet-metal jacket.

The effect of temperature on the iodine value

Samples of almond oil were brominated in the absence of light at 0°C., when vlaues of 100·0 and 100·7 were obtained, showing no appreciable difference due to change in temperature.

METHOD

Reagents

Bromine C.P. grade.
Sodium hydroxide (pellets).

Apparatus

The brominating chamber shown in Fig. 1 consists of an ordinary 26 oz. glass bottle with the bottom removed, both ends being closed by waxed corks fitted with taps. The chamber is divided into two equal sections by a thin glass plate, while the whole is enclosed in a sheetmetal jacket.

The vacuum desiccator is modified by fitting inlet and outlet tubes for the passage of nitrogen or air over the plates.

Glass plates 10 by 5 by 0·1 cm. in size are roughened on both sides by grinding with 100 mesh carborundum.

The carriage is made of 0·2 cm. glass rod and supports the plate only by the four corners to ensure freedom from contact with the oil film.

Procedure

1. *Bromination*

The samples (0·08 to 0·12 g.) are coated on to glass plates by means of the spreader and accurately weighed. They are then placed in the brominating chamber together with a porcelain boat containing about 2 g. of bromine. The chamber is closed and the reaction allowed to proceed for one hour.

2. *Removal of excess bromine*

At the end of this period both taps are opened and the residual bromine vapour displaced by nitrogen or air. The plates are removed and placed in the desiccator,

nitrogen or air being drawn over the surfaces of the plates for 1 to 2 minutes. The desiccator is evacuated for 5 minutes after which air is allowed to enter. These operations are repeated twice more and the plates are then removed and a current of nitrogen or air at 80°C. blown over the surfaces for 5 minutes. The plates are replaced in the desiccator, which is re-evacuated immediately. The films are allowed to cool and then weighed. The procedure is repeated until constant weight is obtained.

The iodine value is calculated from the equation:—

$$\text{Iodine Value} = \frac{126{\cdot}9}{79{\cdot}92} \times \frac{(W_2 - W_1)}{W_1} \times 100$$

$$= 158{\cdot}8 \frac{(W_2 - W_1)}{W_1}$$

Where W_1 = initial weight of oil sample.
W_2 = weight of brominated oil sample.

In conclusion the authors wish to record their thanks to Professor H. Stephen for his interest and encouragement throughout the course of this investigation.

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SOUTH AFRICAN FISH POISONOUS PLANTS

PART I. PRELIMINARY CHEMICAL INVESTIGATIONS OF THE TUBER OF *NEORAUTANENIA EDULIS C.A. SM.*

by

B. L. VAN DUUREN and P. W. G. GROENEWOUD

OPSOMMING

Ses organiese verbindinge is uit die bol van *N. Edulis C.A. Sm.*, 'n Suid Afrikaanse plant, geïsoleer. Hierdie plant, wat in die noordelike Transvaal voorkom, word deur die inboorlinge gebruik om visgif voor te berei. Hierdie verbindinge is ge-ontleed en molekulêre formules word aangegee. Die toksisiteit van die verbindinge vir visse word elders in hierdie tydskrif beskryf.

SUMMARY

Six organic compounds were isolated from the tuber of *N. Edulis C. A. Sm.*, a South African plant occurring in the Northern Transvaal where it is used as a fish poison. These compounds have been analysed and molecular formulae are given. The fish-toxic properties of these compounds appear elsewhere in this Journal.

Certain portions of various tropical plants have been used for centuries as fish poisons. The pulverized plant material is thrown into fish-inhabited waters when the fish die or become temporarily unconscious.

Various fish poisonous plants occur in South Africa. Amongst these is the plant *Neorautanenia Edulis C. A. Sm.*, which is co-specific with *N. Coriaceae C. A. Sm.*This plant occurs in the Northern Transvaal, where its tuber is used by the natives as fish poison.

As far as is known no chemical investigations have yet been conducted on this plant.

The present investigation is concerned only with the tuber of *N. Edulis* since it is this portion of the plant which is used as fish poison.

After preliminary experiments it was decided that the most efficient method of extraction is a process of percolation with ether as solvent.

The ether-soluble material consisted for the greater part of resinous material which is extremely soluble in ether and could hence be readily separated from the crystalline products.

The nature of these products varied from month to month. This variation may be due to seasonal changes and/or differences in the ages of the different tubers extracted.

The extraction of the tubers gave yields varying between 0·56 per cent and 0·70 per cent of crude crystalline material. Due to the variation in the nature of the products of the different extractions, different methods had to be applied for the separation, isolation and purification of the products of the various extractions.

Extraction 1

Preliminary experiments on a part of the product indicated that only one substance could be obtained pure by fractional crystallization, viz., yellow needles M.P. 211°C. The mother liquor contained a mixture of substances of similar solubilities.

Chromatographic adsorption: The following adsorbents were tested: alumina, magnesium oxide and calcium carbonate. Due to the insolubility of the compounds in ether and petroleum ether, only the following solvents were considered: acetone, ethyl acetate, ethylene dichloride and chloroform. Most satisfactory results were obtained with alumina as adsorbent and ethylene dichloride as solvent. For the elution of the various bands the following eluents were used:—

(*a*) Ethylene dichloride for the elution of the most weakly adsorbed substances.
(*b*) Acetone for the more strongly adsorbed substances.
(*c*) A mixture of acetone and alcohol for the most strongly adsorbed substances.

A portion of the adsorbed resins could not be removed from the alumina by any of the eluents tested.

The chromatographic method was applied for the large-scale separation of the substances present in extraction 1. The following substances were obtained:—

Substance (i): Yellow needles, M.P. 211°C.
Substance (ii): Colourless needles, M.P. 251-252°C. (decomp.).
Substance (iii): Light-yellow prisms, M.P. 180°C.

Extraction 2

The following two substances were separated by fractional crystallization:—

Substance (iv): Straw-coloured prisms, M.P. 149°C.
Substance (v): Yellow needles, M.P. 256°C.

By the chromatographic method on the same lines as described above, Substances (i) and (v) were obtained. It was found that Substance (iv) was decomposed on a column of alumina, an orange-coloured resinous product being formed. Hence, for the large-scale separation of the substances in the product from extraction 2 the method of fractional crystallization alone was used. A small amount of Substance (iii) was found to be present in the mother liquor of the crystallizations.

Extraction 3

The product of this extraction contained one substance only, viz. substance (ii).

The yields of the various products are given in Table I below.

TABLE I

Extraction	*Month*	*Per cent yield*	*Substances obtained**	*Approximate yield (as per cent of total pure crystalline material)*
I.	September	0·70	(i)	90
			(ii)	2·5
			(iii)	7·5
II.	February and April ...	0·65	(i)	2·5
			(iii)	2·5
			(iv)	15·0
			(v)	80·0
III.	May	0·56	(ii)	100

* For molecular formulae see Table II below.

A substance of M.P. 230°C. had been isolated in a preliminary small-scale extraction from the tuber of *N. Edulis*. This substance, which crystallizes as colourless needles was not obtained in any of the extractions discussed above. This substance, (vi), was investigated with the others.

Molecular weight determinations and carbon-hydrogen analyses were conducted

for all six substances. From the results the following molecular formulae were calculated:—

TABLE II

Substance	*Molecular formula*
(i)	$C_{18}H_{12}O_6$
(ii)	$C_{15}H_{16}O_6$
(iii)	$C_{19}H_{14}O_6$
(iv)	$C_{17}H_{14}O_5$
(v)	$C_{26}H_{22}O_7$
(vi)	$C_{18}H_{14}O_5$

Various methods were investigated for the removal of crystalline material from the ether-soluble resins:—

(*a*) Adsorption on a column of alumina from solutions in acetone or ethylene dichloride.

(*b*) Precipitation of crystalline material from solutions in ether by addition of alcohol.

(*c*) Repeated extractions with small amounts of ether or acetone at a time.

Only small amounts of crystalline material were obtained from the resins by these methods.

EXPERIMENTAL.

All melting points are corrected.

Method of extraction

The moist tubers, as they were received, were washed with water, cut into small portions and dried in air. When quite dry the material was packed into three percolators, each of which took one kilogram of material. The material was then soaked with ether and the taps so adjusted that the ether percolated through slowly. The ethereal extract was first dark brown in colour, then yellow and finally colourless. When the extraction was complete the greater part of the ether was removed by distillation and the products allowed to crystallize from the ethereal solution (150 ml.). The crude crystalline material was dissolved in a minimum boiling chloroform (100 ml.), ethyl alcohol added until a precipitate formed and just sufficient chloroform added to dissolve the precipitate formed. On cooling, crystallization took place. A further crop of crystals was obtained from the mother liquor.

Separation, isolation and purification

Extraction 1

(*a*) *Fractional crystallization*

The crude crystalline product (5 g.) was crystallized from chloroform (40 ml.) and ethanol (200 ml.) as described above. Yellow needles were obtained which were purified by recrystallization from the same solvent mixture: substance (i), M.P. 211°C.

Found: C, 66·9; H, 3·8. Molecular weight 336
$C_{18}H_{12}O_6$ requires: C, 66·7; H, 3·7%. Molecular weight 324

(b) Chromatographic separation

The crude crystalline product (5 g.) as obtained from the extract, was dissolved in acetone (200 ml.) and poured on a column of activated alumina (100 cm. × 3 cm., prepared from potash alum). The column was eluted with acetone (250 ml.). The solvent was removed by distillation, the residue (4 g.) dissolved in ethylene dichloride (100 ml.) and poured on a column of alumina (100 cm. × 3 cm.). The chromatogram was developed with the same solvent (250 ml.), after which the various bands were well-separated as follows:—

Band	*Colour*	*Section of column.*
1 ...	Dark brown	Top.
2 ...	Orange	—
3 ...	Orange yellow	—
4 ...	Yellow...	Bottom.

The column was then extruded, and cut into four fractions, corresponding to the four bands.

Fraction 1, consisted of adsorbed resins which could not be eluted with any organic solvents and was hence discarded. Fraction 2 was eluted partly with acetone, and partly by extraction in a Soxhlet apparatus with a 1 : 1 mixture of acetone and ethanol for four hours. Fractions 3 and 4 were eluted with acetone. The solvents were removed by distillation and the residues treated as follows:

Fraction 2: Colourless needles, M.P. 251-252°C. (decomp.) crystallized from chloroform and alcohol (Substance (ii)) .

Found: C, 66·4; H, 4·8; Molecular weight 320
$C_{16}H_{16}O_6$ requires: C, 65·85; H, 4·75%; Molecular weight 328

Fraction 3, was rechromatographed on alumina by the same method as described above, when two main fractions were obtained: (*a*) gave on crystallization substance (i), M.P. 211°C.; (*b*) gave on crystallization from ethylene dichloride and ethanol a mixture of yellow needles (substance (i)) and light yellow prisms. These two substances were separated by "handpicking" of the crystals. The light yellow prisms, substance (iii), were purified by vacuum sublimation (0·01 mm./140°C.), M.P. 180°C.

Found: C, 67·4; H, 4·35, Molecular weight 310
$C_{19}H_{14}O_6$ requires: C, 67·45; H, 4·1%, Molecular weight 338

Fraction 4: Substance (i) was obtained pure from this fraction.

Extraction 2

(a) Fractional crystallization

Fractional crystallization gave two products:—
Substance (v), yellow needles, M.P. 256°C.

Found: C, 69·9; H, 4·7, Molecular weight 445
$C_{26}H_{22}O_7$ requires: C, 70·0; H, 4·9%, Molecular weight 446

Substance (iv), light straw-coloured prisms, M.P. 149°C.

Found: C, 68·5; H, 4·5, Molecular weight 329
$C_{17}H_{14}O_6$ requires: C, 68·45; H, 4·7%, Molecular weight 298

The mother liquor left an orange-coloured residue from which a small amount of Substance (iii) was obtained.

(b) Chromatographic separation

The crude crystalline product (0·5 g.) as obtained from the extract was dissolved in ethylene dichloride (15 ml.) and poured on a column of alumina (25 g.). The chromatogram was developed with the same solvent (100 ml.). The following bands were obtained:—

Band	*Colour*	*Section of column*
1 ...	Dark brown ...	Top.
2 ...	Orange yellow	—
3 ...	Yellow ...	Bottom.

After standing overnight (15 hours) Band 2 had broadened considerably and had become darker in colour. Between Bands 1 and 2 a series of indistinct bands were observed. The column was extruded and cut into three fractions corresponding to the three main bands. The fractions were eluted with acetone and the solvent removed by distillation. From Band 2 substance (v) (0·15 g.) was obtained. From Band 3 substance (i) was obtained. Band 1 left a resinous residue.

Extraction 3

Recrystallization of the crude product of this extraction gave a colourless substance, M.P. 251-252°C. (decomp.), undepressed on admixture with substance (ii) obtained from Extraction 1.

Preliminary extraction : isolation of substance (vi)

A colourless substance, M.P. 230°C., was isolated during another season from *N. Edulis*. This substance, (vi), was purified by crystallization from chloroform and ethanol.

Found: C, 69·1; H, 4·1. Molecular weight 324
$C_{18}H_{14}O_5$; requires: C, 69·7; H, 4·5% Molecular weight 310

Decomposition of substance (iv) on alumina

Substance (iv), (0·1 g.) was dissolved in ethylene dichloride (10 ml.) and the solution poured on a column of alumina (10 g.). No coloured bands were observed. After standing for 16 hours the whole column was orange-coloured.

Examination of the resins

(*a*) Precipitation from acetone solution: The resin from extraction 1 (5 g.) was dissolved in boiling acetone (50 ml.). On cooling a yellow deposit formed. This was crystallized from chloroform and alcohol. Substance (i) (0·1 g.), was obtained.

(*b*) Precipitation from ether solution by alcohol: The resin from extraction 2 (25 g.) was dissolved in ether (50 ml.) by boiling under reflux. Alcohol (25 ml.) was added when a crystalline precipitate was formed. This was washed with ether and crystallized from chloroform and alcohol. Substances (iv) (0·08 g.) and (v) (0·1 g.) were obtained.

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SOUTH AFRICAN FISH POISONOUS PLANTS

PART II. DETERMINATION OF THE FISH TOXIC PROPERTIES OF THE ORGANIC COMPOUNDS ISOLATED FROM THE TUBER OF *NEORAUTANENIA EDULIS C. A. SM.*

by

B. L. VAN DUUREN and P. W. G. GROENEWOUD

OPSOMMING

Die toksisiteit van die ses verbindinge wat van die bol van *N. Edulis C. A. Sm.* ge-isoleer is (lees elders in hierdie tydeskrif) is ondersoek. Die toksisiteitskurwe van een van die stowwe is opgestel en met die van rotenoon vergelyk. Die toksisiteit van die verbinding is van dieselfde orde as die van rotenoon.

SUMMARY

The six organic compounds isolated from the tuber of *N. Edulis C. A. Sm.* (see elsewhere in this journal) were examined for fish toxic properties. Two of these compounds were found to be fish toxic. The toxicity curve of one of these substances was constructed and compared with that of rotenone constructed under identical experimental conditions. The toxicity of this substance was found to be comparable with that of rotenone.

The toxicity determinations were conducted for the following two reasons:—

(*a*) To compare the fish toxic properties of the "new" substances with that of well-known fish poisons.

(*b*) To secure data by which a correlation between toxicity and chemical constitution may eventually be made.

Due to the insolubility of the compounds under investigation in water, the following two methods were attempted for obtaining colloidal suspensions in the water:—

(*a*) By dissolving the substance under examination in acetone and adding this solution to water. This method was used by Gersdorff[1] in the determination of the toxicity of rotenone.

(*b*) Takei *et al.*[2] used a soap-olive oil emulsion of rotenone in the determination of its toxicity.

Both methods were found unsatisfactory for the compounds under discussion. The most suitable procedure was found to be the addition of an acetone solution of the substance under investigation to the correct volume of water containing a protective colloid. For the latter purpose soap, gelatin or agar-agar was used. Control experiments showed that these protective colloids had no ill-effects on goldfish, provided, however, that suitable concentrations as recorded below were employed.

Goldfish (*Carassius auratus*) were used in all the toxicity determinations.

Of the six substances isolated, four were found to have only slight irritating effects on goldfish. The other two substances, $C_{17}H_{14}O_5$, and $C_{19}H_{14}O_6$, M.P. 149°C. (corr.) and 180°C. (corr.) respectively, were found to be definitely toxic to goldfish.

The effect of the fish poisons were found to decrease with time. This may be due to separation of the poison from colloidal suspension. Therefore the fish were placed in fresh poison "solutions" at regular intervals during toxicity determinations.

The toxicity curve of rotenone was constructed and compared with that given by Gersdorff (*loc. cit.*). The toxicity curve of the one of the "new" fish poisons was then constructed under exactly identical experimental conditions.

EXPERIMENTAL

Control experiments with protective colloids

Goldfish of weight 2·5 to 3·0 g. were kept in each of the following protective colloids without any apparent ill-effect:—

(*a*) Soap (0·0001 per cent., 500 ml., prepared from dry alkali-free soap).
(*b*) Gelatin (1·0 per cent, 500 ml.).
(*c*) Agar-agar (0·2 per cent, 500 ml.).
(*d*) Soap (0·0001 per cent, 250 ml.) and agar-agar (0·2 per cent 250 ml.).

Pure distilled water which had been aerated for three hours was used in these experiments. Each solution contained in addition the maximum permissable amount of acetone (0·5 ml. in 500 ml. water).

Toxic properties of the new substances

Saturated solutions in acetone were prepared for the six substances. The six acetone solutions (0·5 ml.) were added to six gelatin solutions (500 ml., 1·0 per cent) each containing one goldfish (2·5 - 3·0 g.) at room temperature (23°C.) and the temperature raised over a period of three hours to 27°C. and kept constant at 27$\pm$0·25°C. The fish in the gelatin solutions containing substances (i), (ii), (v) and (vi) (see Part I of this series for molecular formulae) appeared to be slightly affected in the beginning but the effect decreased after 45 to 60 minutes and the substances then had no ill-effect even after 24 hours. The fish in the solutions containing substances (iii) and (iv) were both badly affected after 30 minutes and both were dead within two hours.

The experiments were repeated with substances (i), (ii), (v) and (vi); the fish, however, were placed in fresh poison "solutions" after each hour for three hours running. Again the apparent ill-effects were only temporary.

Aerated distilled water was used in these experiments.

The toxicity curve of rotenone

Goldfish of weight varying between 2·5 and 3·0 g. were each placed in aerated distilled water (500 ml.) at room temperature (23°C.) and the temperature gradually raised to 27°C. over a period of three hours. Freshly prepared solutions of rotenone of various strengths in acetone (0·5 ml.) were added to the water containing the fish and the temperature kept constant at 27$\pm$0·25°C. The survival times of the fish were then taken. This is the period between the addition of the poison and the death of the fish. The death point was taken when there was no movement of mouth or gills for 60 seconds. The results are given in Table I below.

From these results the *survival time curve* of rotenone was constructed. (Fig. 1). The ordinate represents the survival time in minutes and the abscissa concentration in milligrams per litre.

The toxicity curve of substance (iv)

The same experimental conditions were used as were used in the construction of the rotenone curve except for the addition of protective colloids in the present case and the fact that the fish were placed in fresh poison "solutions", at the same concentrations and temperature after each hour during toxicity determinations. The results are given in Table II below.

TABLE I

Concentration (milligrams per litre)	*Weight of fish (grams)*	*Survival time (minutes)*	*Mean survival time (minutes)*
4	2·6	52	53
	2·5	54	
2	3·0	53	53·5
	2·8	54	
1	2·5	55	55
	2·7	55	
0·2	3·0	84	81
	2·6	78	
0·1	3·0	125	120
	3·0	116	
0·05	2·7	154	159
	2·8	164	
0·025	2·7	484	487
	2·9	490	

TABLE II

Concentration (milligrams per litre)	*Weight of fish (grams)*	*Protective colloid*	*Survival time (minutes)*	*Mean survival time (minutes)*
20	2·9	1 per cent gelatin	117	117
15	3·0	1 per cent gelatin	121	121
10	2·5	1 per cent gelatin	124	124
7·5	2·8	0·5 per cent gelatin	153	150
	2·6	0·5 per cent gelatin	147	—
5·0	2·5	0·5 per cent gelatin	215	221
	2·9	0·5 per cent gelatin	227	—
4·0	2·7	0·2 per cent agar-agar + 0·0001 per cent soap	260	260
	2·9	0·2 per cent agar-agar + 0·0001 per cent soap	273	
2·5	2·8	0·2 per cent agar-agar + 0·0001 per cent soap	382	390
	2·9	0·2 per cent agar-agar + 0·0001 per cent soap	398	

From these results the survival time curve of substance (iv) was constructed, (Fig. 2).

INTERPRETATION OF THE RESULTS

The toxicity curve for rotenone given above is almost identical with that obtained by Gersdorff[1]. The slight displacement can be accounted for by the fact that the fish used by Gersdorff were somewhat smaller than those used in the present investigation.

There is as yet no formula or method by which the toxicities of substances can be compared in such a manner that all the essential factors are taken into account. The following methods have been used by various workers:—

I. Comparison of survival times at a given concentration.

II. Comparison of the concentrations necessary to cause death (or any other phenomenon used as the criterion) in any arbitrarily fixed time.

III. Comparison of toxicities by applying the formula of Powers (see below).

IV. Comparison of the rates of increase of velocity of fatality with increase in concentration.

V. Comparison of the concentrations necessary to just cause death (called the threshold of toxicity).

VI. Comparison of the minimum survival times. The latter is the survival time in the region of constant velocity of fatality, i.e., that part of the survival time curve that approaches the horizontal.

It is clear, therefore, that the values for the relative toxicities will differ according to the criterion used. This fact becomes quite clear from a study of the toxicity curves constructed by Gersdorff.[1, 3-6]

Gersdorff, to whom much of the pioneer work on the determination of the toxicities of various substances is due, later on discarded methods I, II and III above and gave his results in terms of the last three methods mentioned above. This procedure seems justified in view of the following considerations:—

(*a*) A study of the survival time curves shows that the relative toxicities will depend upon the concentration at which they are compared. Similarly the toxicities will not be the same when the concentrations necessary to cause death in different arbitrarily chosen survival times are compared.

(*b*) The formula of Powers[7] does not include all the factors determining toxicity.

Toxicities can best be compared by a study of the *velocity of fatality curves*. This type of curve is obtained when the toxicity of a substance at various concentrations is plotted in such a manner that the ordinate represents the reciprocal of the survival time x 100 and the abscissa represents concentration in milligrams per litre. From this type of curve it was observed that there were at least two independent variables in toxicity:—

(i) The threshold of toxicity, i.e., the concentration just sufficient to cause death.

(ii) The rate of increase of velocity of fatality with increase in concentration.

Powers[7] adopted as a measure of toxicity a value based on the reciprocal relation of these two factors and calculated the toxicity (T) of a substance from the following formula:—

$$T = \sqrt{\operatorname{Tan}\,\theta/\alpha}$$

where: Tan θ represents the slope of that part of the curve that approaches a straight line (i.e., the rate of increase of velocity of fatality with increase in concentration):

α represents its point of intersection, when produced, with the X-axis (i.e., the threshold of toxicity).

This formula, however, does not necessarily include all the factors of toxicity and hence the two variables as depicted in the velocity of fatality curves will be discussed separately.

(*a*) The rate of increase of velocity of fatality with increase in concentration (method IV alluded to above) is obtained from the slope of that portion of the curve that approaches a straight line. From the velocity of fatality curves of substance (iv) and rotenone (Figs. 3 and 4 and Tables III and IV) it is seen that if this criterion is used substance (iv) appears to be much less toxic than rotenone.

(*b*) The comparison of toxicities by using the concentrations just sufficient to cause death, i.e., the threshold of toxicity, again involves only one factor of toxicity. By comparison of the two curves (Figs. 3 and 4) it is seen that the threshold of toxicity of the two substances is of the same order. This fact is significant if it is remembered that nicotine and anabasine become toxic only at considerably higher concentrations, viz., 8·0 and 9·5 milligrams per litre respectively,[5] whereas the corresponding values for rotenone and substance (iv) are of the order of a fraction of a milligram.

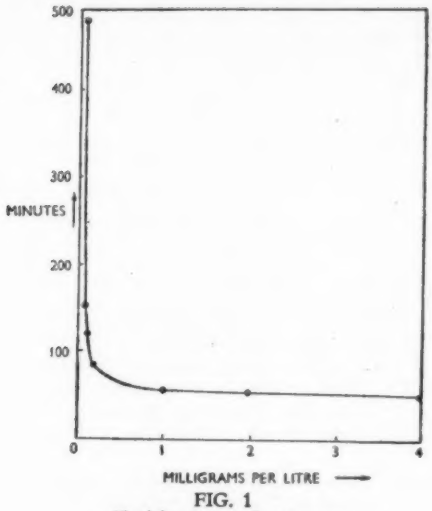

FIG. 1
Toxicity curve of rotenone.

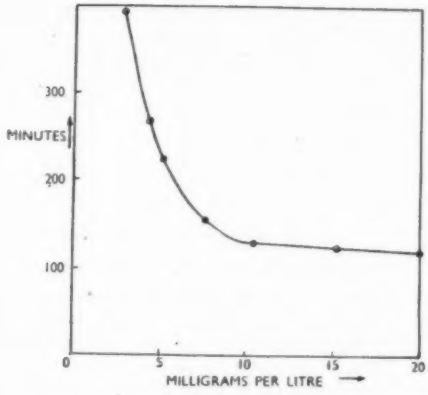

FIG. 2
Toxicity curve of substance (iv) ($C_{17}H_{14}O_6$).

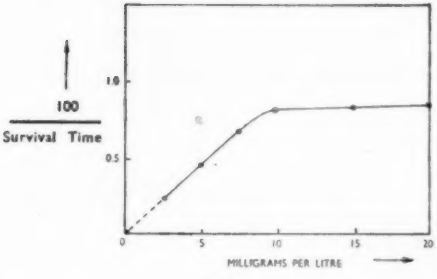

FIG. 3

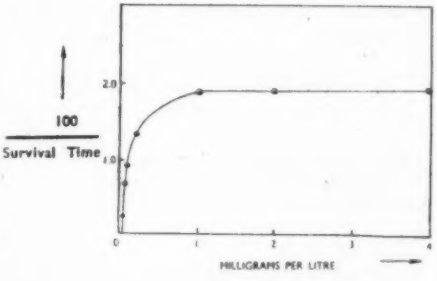

FIG. 4

TABLE III
Data for the velocity of fatality curve of substance (iv)

Concentration (*milligrams per litre*)	*Mean survival time* (*minutes*)	$\frac{100}{\text{Mean survival time}}$
20	117	0·85
15	121	0·83
10	124	0·81
7·5	150	0·67
5·0	221	0·45
4·0	266	0·37
2·5	390	0·26

TABLE IV
Data for the velocity of fatality curve of rotenone

Concentration (milligrams per litre)	*Mean survival time (minutes)*	$\frac{100}{\text{Mean survival time}}$
4	53	1·88
2	53·5	1·87
1	55	1·81
0·2	81	1·23
0·1	120	0·83
0·05	159	0·62
0·025	487	0·20

Gersdorff, in his later papers[3–5] regarded the minimum survival time method (method VI) for the comparison of toxicities as one of the most important methods. The minimum survival time is obtained directly from the survival time curve, viz., that portion of the curve at the higher concentrations, which becomes practically a horizontal line. This is assumed by Gersdorff[4] to occur when the survival times corresponding to concentrations one of which is double that of the other do not differ by more than 5 per cent.

According to this method the minimum survival time in substance (iv) is 115 minutes, which is comparable with that of rotenone as calculated by Gersdorff.[5] Comparison with the two alkaloids nicotine and anabasine is also interesting if it is taken into consideration that they become toxic at higher concentrations than substance (iv) and rotenone. The minimum survival times in the two alkaloids are 20 and 22 minutes respectively.[6]

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THE AUTOCATALYTIC GROWTH CURVE

PART I. INCREASE IN WEIGHT OF INFANTS

by

P. R. v.d. R. COPEMAN

OPSOMMING

Die verband tussen veranderings in die groei en die tydfaktor kan, in die geval van die dierlike organisme uitgedruk word met behulp van die vergelyking:—

$$\log (x + k_0)/(a - x) = (k_1 + k_2 a)(t - t_0) \text{ wanneer } k_0 = k_1 k,$$

Deur hierdie uitdrukking toe te pas op gegewens in verband met die groei van kinders is aangetoon dat die konstantes "k_1" en "k_2" beskou kan word as konstantes wat respektiewelik afhanklik is van eksterne en interne faktore.

"k_1" is afhanklik van eksterne faktore en dit blyk dat 'n hoë waarde vir k_1 gepaard gaan met 'n laë waarde vir "a".

"k_2" is afhanklik van die ras en geslag en kan beskou word as 'n spesefieke, inherente konstante van die organisme.

SUMMARY

The changes of growth with time in the case of animal organisms may be suitably expressed by the equation:—

$$\log (x + k_0)/(a - x) = (k_1 + k_2 a)(t - t_0) \text{ where } k_0 = k_1/k,$$

By the application of this expression to the data for the growth of infants it has been shown that the constants "k_1" and "k_2" can be regarded as constants associated with external and internal factors respectively.

"k_1" is affected by external conditions and it appears that a high value of "k_1" is associated with a low value of "a".

"k_2" is dependent upon race and sex and may be regarded as a specific inherent constant of the organism.

Growth of infants.

Many efforts have been made to formulate quantitatively the changes with time which occur during the growth of animals and plants. Frequently more than one cycle of development may be apparent during the total life of the organism. As a rule, in any given cycle of growth the accumulation of material is slow in the initial stages. This stage is succeeded by a period of rapid growth which is followed by a subsequent slackening of growth. This last stage is coincident with the approach of maturity for the particular cycle of growth under consideration. In these circumstances the curve of growth with time is sigmoid in character and can be expressed by means of an equation for an autocatalytic reaction. This has led to the assumption that such growth processes are autocatalysed. This conception has been vigorously developed by many workers, notably by Robertson[11] who has applied this idea particularly to the growth of animals. Although the growth process may be an extremely complex phenomenon it is assumed that the changes associated with growth can be regarded as being dependent upon a "master reaction" which can be represented by the equation for a monomolecular autocatalytic transformation. Some measure of success has also followed the application of this idea, to express the changes with time which occur in the case of plants. For example, Gregory[8] has shown that the changes in the lengths and in the areas of the leaves of *Cucumis sativus* with time may be readily expressed by means of such an equation. Reed and Holland[10] used the same equation to express the increase in height of *Helianthus* and showed that the deviations from the

formula were not significant. The author [2,4] has used the equation to express the changes which occur in various constituents of grapes and oranges during ripening. Gaines and Nevens[7] have also developed this idea in a study of the changes with time of various constituents in sunflower and maize. If the constants of the equation have the significance attached to them by Robertson, they should serve a useful purpose in giving a numerical expression to the course of the growth changes and be of value in supplementing the usual data of crop-yield.

Robertson[11] has developed the autocatalytic equation for growth in the form:

$$\log x/(a-x) = K(t-t_0) \quad \ldots\ldots(1)$$

where a = maximum yield of material at the completion of the growth cycle, x = amount of material at time t, t_0 = time when x = a/2 and the rate of growth is at a maximum, K = constant.

The velocity of growth is given by—

$$dx/dt = kx\,(a-x) \quad \ldots\ldots(2)$$

where K = ka, k being the velocity constant of the change.

The expression in the form of equation (1) is suitable for application to observed data, and Robertson[11] has applied it in this form to the data for the increase in weight of infants during the first year after birth. The data for the monthly weights of male and female infants were compiled from the records of institutions in England (London and Leeds), in South Australia (Adelaide) and in South Germany (Frankfurt), and represent the normal changes in weight for infants from these localities. Any weights which might be regarded as abnormal owing to illness, etc., were excluded in calculating these average figures. The constants, as determined by Robertson in accordance with equation (1), are given in Table I in which "a" is reported in ounces and "t_0" in months from the time of birth, using logarithms to the base 10.

TABLE I

	a	*K*	*k*	t_0
	Male infants			
British	318	0·127	0·000399	1·40
South Australian ...	341·5	0·136	0·000398	1·66
South German	314·6	0·142	0·000451	2·65
	Female infants			
British	312	0·106	0·000340	1·54
South Australian	350	0·111	0·000317	2·47
South German	290	0·156	0·000537	2·25

According to Robertson, "a", the constant representing the maximum growth attainable during the cycle of growth, is expressive of the total mass of available nutrient material and is dependent upon the average concentration of nutritional material in the tissues. It may be expected that conditions, favourable to the growth of an organism will result in a high value for "a" and therefore that "a" is, to a considerable degree, subject to modification by environmental conditions. Robertson maintains that "a" is not greatly affected by sex or race since the values obtained in the similar environments of London–Leeds and of Frankfurt are "very much alike" but that it is greatly affected by dissimilarities in environment as shown by the values of "a" for Australia and

Europe. In this connexion it is pointed out that the evolution of the Australian people must be regarded as that of British people under changed climatic, social and economic conditions. The data cannot be due to any racial selection among the immigrants from the British Isles. On the other hand the constant "k" is regarded as a specific velocity constant, internal in character and expressive of the intimate nature of the growth-process itself and independent of the nutritional level of the tissues. In support of this view it is pointed out that the value of "k" is practically identical for British males in England and in Australia and very nearly the same for British females in these two environments, while "k" is profoundly affected by sex and race as indicated by the differences between the values of "k" for males and females, and between British and South German infants.

A closer inspection of the results in Table I will show, however, that the above conclusions are not entirely valid. For example, the values of "k" for British and Australian female infants are not in such close agreement as the corresponding values for male infants. In addition, the differences in the value of "k" between South German male and female infants are not in the same direction as the differences for British males and females. Since K=ka, it is clear that it is not possible to separate the effects of internal and external factors on the growth of the organism. "a" must be the effects of internal and external factors on the growth of the organism. "a" must be regarded as the resultant of the combined action of these factors. In the case of plants a high value of K indicates a short growing-period and quite naturally a short growing-period tends to be associated with a low crop-yield, i.e., with a low value of "a". The value of "k" will therefore be subject to variations in accordance with external conditions. For example, Prescott's data[9] for the flowering curve of Egyptian cotton show that "k" is subject to such variations. In the case of grapes the author[8] has shown that changes in locality cause distinct and definite changes in the value of "k". If the nutritional level in the tissues is maintained at a high level the value of "a" will tend to be high and, since K=ka, the value of K may also be high and small changes in the value of "k" obscured. It must be supposed that the evolution of the higher types of animal organisms has been accompanied by an increased perfection of the various mechanisms required to maintain constancy of the "nutrient level." In cases where the concentration of nutritional materials is altered by changes in the external conditions it must be expected that the value of "k" will vary accordingly. The results obtained in practice with a large number of organisms all tend to show that "k" is not a specific inherent constant, independent of external conditions.

The conception of a specific inherent constant is an attractive one and it is worth considering whether it might not be possible to evaluate such a constant and to determine separately the effects due to the internal and external conditions which determine the course of development of an organism. Crozier[6] has pointed out that the curve represented by equation (1) assumes that the growth during the cycle of development is symmetrical about a mid-point. In these circumstances the temperature characteristic of the velocity constant "K", must be constant throughout any cycle of growth. There is adequate information that such may not be the case. Brown[1] has shown that an alteration in the temperature of the environment causes an alteration in the curve of growth of an organism such that the two curves will not coincide along the course of development. Robertson[11] has attempted to overcome the lack of symmetry by supposing that the curve may be modified by the fusion of two cycles of growth. If however, the equation describing growth has more than one velocity constant and these constants are unequally modified by external conditions, changes in the curve may be capable of direct interpretation. It may be supposed that during development,

the formation of material is due to a first order transformation in which the product formed serves as a catalyst for the change. The reaction, therefore, will be governed by a velocity constant "k_1" proper to it in the absence of x, and also by a velocity constant "k_2" due to catalysis by x. The change, therefore, must be considered as due to two parallel reactions and the total rate of change will be given by—

$$dx/dt = (k_1 + k_2x)(a-x) \quad \ldots\ldots(3)$$

where a = maximum material at the end of the cycle of growth,

k_1 = velocity constant of the first order transformation,

k_2 = velocity constant of the change governed by the catalytic effect of x.

The integral of this equation is—

$$\log(k_2x+k_1)/k_2(a-x) = (k_1+k_2a)(t-t_0) \quad \ldots\ldots(4)$$

where "t_0" is the time of maximum rate of growth when—

$$x=(k_2a-k_1)/2k_2.$$

If $k_0=k_1/k_1$, the equation may be written:

$$\log(x+k_0)/(a-x) = (k_1+k_2a)(t-t_0) \quad \ldots\ldots(5)$$

and t_0 = time when $x = (a-k_0)/2$.

The expression in the form of equation (5) is suitable for application to observed data.

If any change of condition, e.g., temperature, influence "k_1" and "k_2" unequally, the form of the curve connecting x with time will be changed and the point of inflexion changed to a new position. If "k_1" is of inappreciable magnitude, the curve will be practically the same as that used by Robertson. However, since this modified equation of growth (equation (5)) has two velocity constants, it will be modified by changes in external conditions and the curve of growth will not be coincident at all points when it is brought into agreement at its end points with the curve of growth described by equation (1). It must be expected that the velocity constant "k_1" will be directly affected by changes in external conditions and may be regarded as a measure of these variations, and therefore as an "external" constant. On the other hand, the constant "k_2" must be dependent upon the concentration of transformable material present in the tissues during growth and must therefore have the properties of an "internal" constant. Since this factor is likely to be an inherent characteristic of an organism the constant "k_2" will also be a specific constant for the organism.

On comparing the constants in equation (1) for the growth curve with equation (5) it will be seen that, as an approximation—

$$ka=k_1+k_2a \text{ or } k=k_1/a+k_2 \quad \ldots\ldots(6)$$

Since a high value of k_1 will be characteristic of a short-growing period, the value of "a" will be correspondingly small. In these circumstances, k_2 may remain substantially constant while the value of "k" will vary inversely with "a". If "a" is large and the variations in "k_1" are small, k may remain practically constant. It seems that Robertson's conclusions regarding the specific nature of "k" may be explained in this way but it is clear that, unless the constant "k" be separated into two factors, the effects due to environmental changes may be easily masked.

In a paper on "Autocatalysis and Growth"[5] the author attempted to develop this idea, using Robertson's data for British and South Australian male infants. The results were very significant but a more thorough analysis of these data has now been made and the constants for the growth changes have been recalculated by applying the form of equation (5) to the six sets of data, as reported by Robertson. The results

are given in Table II in which the weight "a" is given in ounces and the time "t_0" in months from the time of birth. Logarithms to the base 10 were used in all the calculations.

TABLE II

	Male infants				
	a	k_1+k_2a	k_1	k_2	t_0
British	320	0·1280	0·0096	0·000370	1·10
South Australian	343	0·1320	0·0051	0·000370	1·35
South German	314	0·1368	0·0028	0·000427	2·45
	Female infants				
British	310	0·1106	0·0051	0·000340	1·35
South Australian	345	0·1172	0·0007	0·000340	2·15
South German	290	0·1360	0·0243	0·000385	1·10

The agreement between the observed and calculated results is shown in Figs. 1–3.

In order to compare the closeness of fit between the observed and calculated data in the case of the autocatalytic curve of equation (1) with that in the case of the modified curve of equation (5), the deviation $\triangle$, the sum of the squared deviations $\triangle^2$, and the value of R in the test for "goodness of fit" as described by Yule and Kendall[12] are given in Table III.

TABLE III

	Male infants					
	Equation (5)			*Equation* (1)		
	$\triangle$	$\triangle^2$	R	$\triangle$	$\triangle^2$	R
British	+1·0	147·0	0·9977	+12·0	154·0	0·9975
South Australian	+3·0	221·0	0·9980	+10·0	220·0	0·9980
South German	−2·0	610·0	0·9927	− 7·0	719·0	0·9837
	Female infants					
British	0·0	100·0	0·9982	− 9·0	147·0	0·9972
South Australian	+17·0	1453·0	0·9867	+58·0	1492·0	0·9863
South German	−16·0	193·0	0·9973	−41·0	455·0	0·9938

It will be noted that in all cases the total deviation in the case of equation (5) is less than that in the case of equation (1). The sum of the squared deviations is also less for equation (5) in all cases except in the case of the South Australian male infants where the sums are practically identical. The value of R is greater in all cases for equation (5), indicating a better fit for this equation except in the case of the South Australian male infants where the values of R are identical. These results indicate that the modified curve of growth as given by equation (5) expresses more closely the changes in growth than the curve of equation (1).

The results given in Table II for the constants are distinctly interesting and some very significant conclusions can be drawn from them. The final weight "a", associated with this cycle of infant growth, is greater for South Australian infants than that for either British or South German infants and it must be concluded that the more favourable conditions for growth in South Australia have had a direct influence upon the value of "a". The values of "a" obtained for infants in the similar environments of London–Leeds and of Frankfurt are more closely related. At the same time the value of "a" appears to be dependent upon sex since, with the exception of South Australian infants, it is larger for male infants than for female infants. Such a conclusion might be expected as a normal result for the growth of infants. In addition the value of "a" appears to be affected by racial factors, since the values for South German infants is

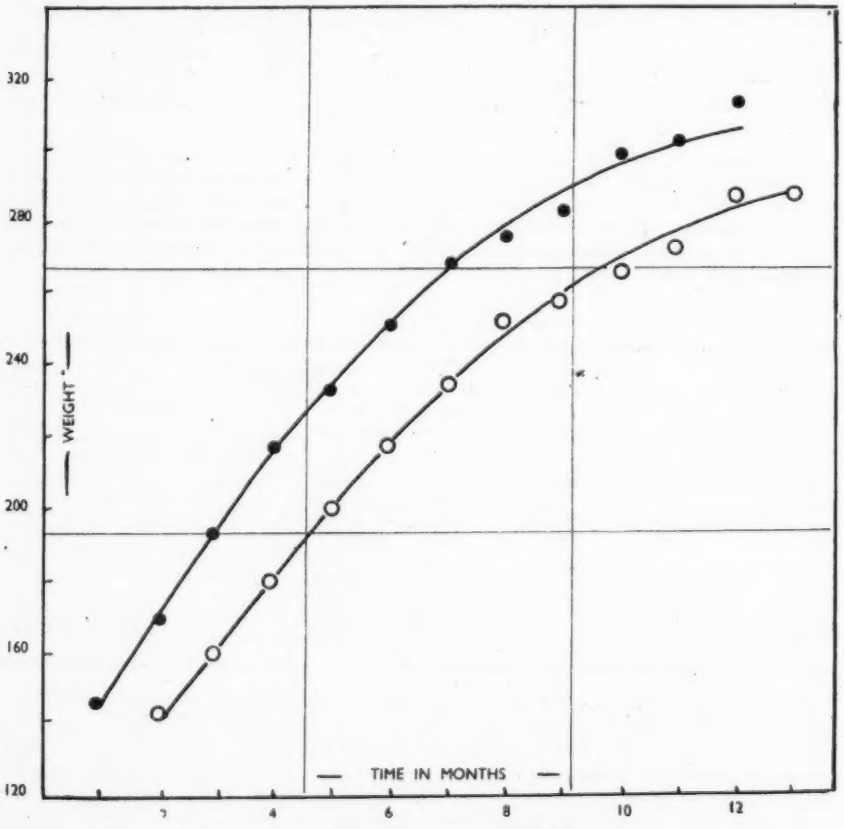

FIG. 1. GROWTH OF BRITISH INFANTS

● = Male. O = Female. (Curve moved by addition of one month to observed times.)

less than for British infants although the environments are very much alike. It must be concluded that the value of "a" can be influenced by both internal and external factors, and must be a resultant of their combined effects upon the course of growth.

The value of the constant, k_1+k_2a, cannot be clearly analysed, since it is obviously dependent upon two factors. In the present case of infants the value is clearly affected by the dominating influence of the factor represented by the quantity "k_2a". This would indicate that the nutritional levels of the tissues tend to be maintained at a normal level in annual organisms despite changes in the external conditions provided that the latter are not far removed from the optimum. It appears to be influenced by sexual factors since, in all cases, the value of "k_1+k_2a" is less for females than for males.

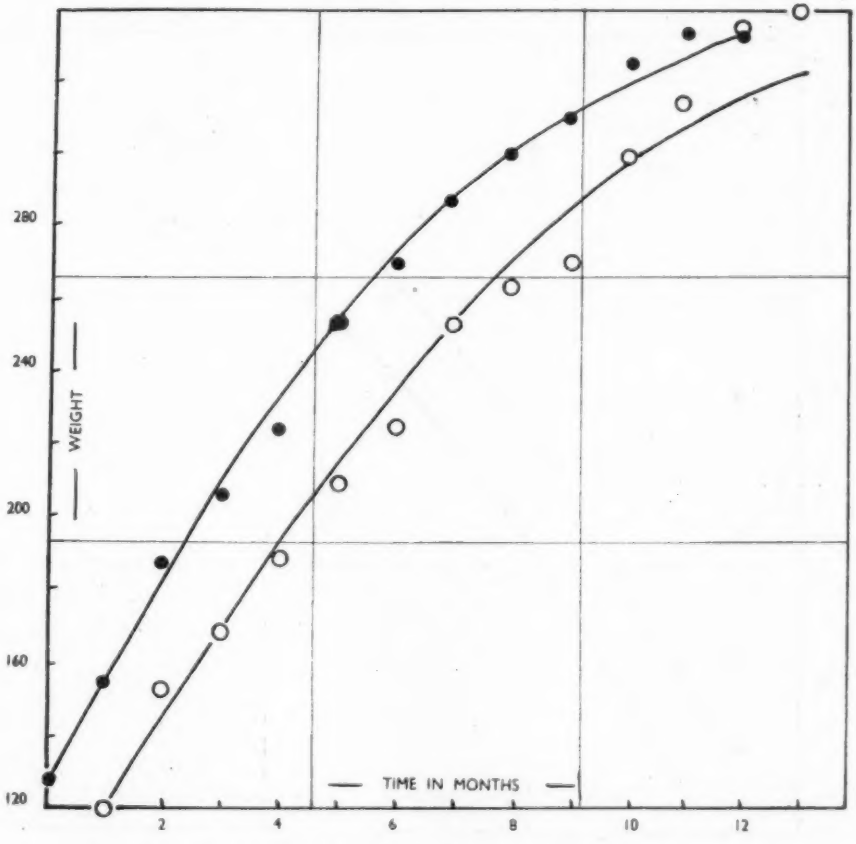

FIG. 2. GROWTH OF SOUTH AUSTRALIAN INFANTS
● = Male. O = Female. (Curve moved by addition of one month to observed times.)

It will be noted that the value of the velocity constant "k_1" is directly dependent upon environment and, as might be expected, a high value of "k_1" is associated with a low value of "a". This is in accordance with the conception that the constant "k_1" is directly affected by changes in external conditions. In the case of British and South Australian infants the value of "k_1" is lower in the latter, indicating a more favourable environment for growth. The constant "k_1" may thus be regarded as an "external" constant.

The values for the velocity constant "k_2" are of considerable interest and a comparison of the values in Table II indicated clearly that this constant is more dependent upon internal factors than the value of "k" as calculated by Robertson. The value of "k_2" is identical for British and South Australian male infants, namely 0·000370, and for the female infants namely 0·000340. These values differ from the corresponding

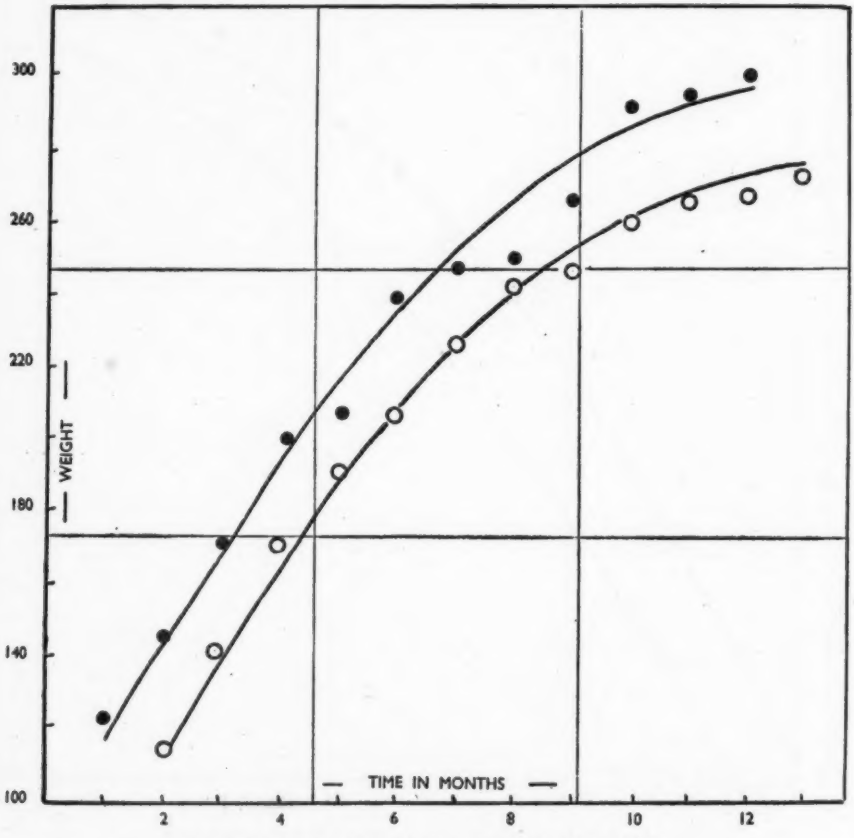

FIG. 3. GROWTH OF SOUTH GERMAN INFANTS
● = Male. O = Female. (Curve moved by addition of one month to observed times.)

values of k_2 for South German infants. In all cases the value of k_2 is higher in the case of the male infant. South German infants are characterized by a higher value for "k_2" as compared with that for British and South Australian infants. However, the results indicate clearly that the constant "k_2" may be regarded as a specific inherent constant which is influenced by such factors as race and sex, and independent of external conditions.

The value of "t_0" indicates the period of maximum rate of growth after birth. In the case of British and South Australian infants, this stage is reached at an earlier date in the males than in the females, but the reverse is the case in South German infants. The period at which this maximum growth rate occurs is dependent upon the relation between the velocity constants "k_1" and "k_2". Where conditions are not so favourable, i.e., where the value of "k_1" is increased, this stage is reached at an earlier date.

It is clear from a comparison of the results in Tables I and II that the constant "k_2" has a much greater significance as a specific inherent constant than the value of "k" as calculated from equation (1). It is affected by race and sex and, from this point of view, should possess a special value in studies of race and variety in animals and plants. At the same time the constant "k_1" is directly affected by external conditions, being lower under conditions which favour the growth of the organism. There can be no doubt that the value of "k" in equation (1) does not have the specific significance attached to it by Robertson and other workers. From the approximation $k=k_2/a+k_2$ it can be seen how the effects of external variations may be masked if the "simple" value "k" only be considered. The nutritional level in the tissues in the case of animals must be less susceptible to variations in external conditions than in the case of plants and therefore it must be expected that the constant "k_2" would possess a greater genetic racial significance than the simple constant "k" which is dependent upon the resultant effect of more than one operational factor. The velocity constants "k_1" and "k_2", therefore, can be used in growth studies to distinguish between the effects of external and internal factors upon the growth of the organism.

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THE AUTOCATALYTIC GROWTH CURVE

PART II. INCREASE IN HEIGHT OF OATS

by

P. R. v.d. R. COPEMAN

OPSOMMING

Die toename in die hoogte van hawer met betrekking tot die tydfaktor kan uitgedruk word met behulp van die volgende vergelyking:—

$\log (x-k_0)/(a-x) = (k_1+k_2a)\,(t-t_0)$ wanneer $k_0 = k_1/k$.

Die hawer is gekweek met agt verskillende kunsmisbehandelings en dit is aangetoon dat, oor 'n tydperk van 8–9 weke, daar drie verskillende periodes van ontwikkeling is.

Dit blyk dat k_2 die eienskappe besit van 'n spesefieke inherente konstante wat onafhanklik is van eksterne faktore. Vir die tweede en derde (groei) periodes het k_2 respektiewelik die waardes 0·00658 en 0·0126 gehad.

Dit is aangetoon dat k_1 varieer met eksterne faktore en dit kan beskou word as 'n eksterne konstante

SUMMARY

The increase of height with time in the case of oats can be expressed by means of the equation:—

$\log (x+k_0)/(a-x) = (k_1+k_2a)\,(t-t_0)$ where $k_0 = k_1/k$.

The oats were grown under eight different fertilizer treatments and it has been shown that, during a period of 8–9 weeks, there were three cycles of development.

It appears that "k_2" has the properties of a specific inherent constant, independent of external conditions. For the second and third cycles "k_2" had values of 0·00658 and 0·0126 respectively.

It has been shown that "k_1" varies with external conditions and can be regarded as an external constant.

In a previous paper on this subject[4] an effort was made to determine constants for the curve of growth with time which would measure the effects of external and internal conditions upon the growth changes. The application to the observed data of a modified autocatalytic equation, evolved by Crozier[6] was found to give constants which were of definite significance from this point of view. The data which were used were those published by Robertson[8] for the increase of weight with time in the case of British, South Australian and South German infants. This worker has developed the simple autocatalytic equation to describe these changes in the form of the following equation—

$$\log x/(a-x) = K(t-t_0) \text{ where } K=ka \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where a = maximum weight attained by the organism during the growth cycle,
x = weight at time t, and t_0=time when $x=a/2$ and k=velocity constant of the change.

Robertson concluded that "a" was a constant depending upon external conditions, while k was regarded as a specific inherent constant, characteristic of the organism. It is obvious, however, that "a" must be a resultant of the combined effects of internal and external conditions, and it has been shown that "k" is also dependent upon external conditions.[3]

In an effort to overcome these difficulties the modified autocatalytic equation was applied to the data reported by Robertson in order to ascertain whether it might not be possible to account separately for the effects of external and internal factors upon the course of growth of an organism. This equation takes the form:

$$\log(x+k_0)/(a-x)=(k_1+k_2a)\,(t-t_0) \text{ where } k_0=k_1/k_2 \quad \ldots\ldots\ldots\ldots (2)$$

where a = the final weight attained during the cycle of growth,
x = weight at time t_1,
t_0 = time when $x=(a-k_0)/2$,
k_1 = velocity constant of the first order transformation, and
k_2 = velocity constant of the reaction catalysed by x.

It has been shown[5] that, in the case of infants, the constant "k_1" was affected in an inverse manner by external conditions, while "k_2" had the properties of a specific inherent constant, characteristic of the organism and independent of external conditions. The results were sufficiently significant to justify these conclusions and it was felt that an effort should be made to ascertain whether similar conclusions could also be applied to plants.

An attempt along these lines has already been published[4] in the case of the changes in the sugar content of grapes during ripening[2] and in the case of the data reported by Prescott[7] for the flowering curve of Egyptian cotton. These preliminary results were significant and tended to show that in the case of plants k_2 might be regarded as a specific inherent constant, while "k_1" was affected inversely by external conditions. Under favourable conditions, as indicated by a high value for "a", the value of "k_1" was low. The values of "k_1" and "k_2" should thus have a distinct value in growth studies of various organisms.

However, it was felt that a more thorough investigation of this idea was required and that a more careful study of the growth changes in plants would be of value. The opportunity of obtaining suitable date for such a study was provided when a series of pot-experiments was started in connexion with the comparative fertilizing values of certain phosphatic products from different sources. The crop used in these experiments was oats, and a number of pots was included in which the oats in each pot received a different fertilizer treatment. The growth changes were followed by measuring the height of the oats at regular intervals. Since all the factors, except fertilizer treatment, were common to all the pots, any differential effects due to external factors could be ascribed to the variations in fertilizer treatment and each pot would serve as a control for each of the other pots in the case of variations in other external factors such as temperature, humidity, etc. For this reason it was felt that it was not essential to attempt a strict control of all possible external factors which might influence the growth of the crop. The work involved would not be commensurate with the object in view, namely, a general application of the views outlined above.

Eight pots were used, each being filled with 12 Kg. of a well-mixed uniform sample of soil, spread over a layer of small stones to provide adequate drainage. The following treatments were used:—

I	...	...	...	...	...	...	O
II	...	...	...	...	...	...	NK
III	...	...	...	...	...	...	NP
IV	...	...	...	...	...	...	PK
V	...	...	...	...	...	...	NPK
VI	...	...	...	...	...	...	N_2PK
VII	...	...	...	...	...	...	NPKCa
VIII	...	...	...	...	...	...	N_2PKCa

where O = no fertilizer;
N = nitrogen at the rate of 300 lb. of ammonium sulphate per acre;
P = phosphate at the rate of 600 lb. of 20 per cent water-soluble superphosphate per acre;
K = potash at the rate of 100 lb. of potassium sulphate per acre;
Ca = lime at the rate of 4,000 lb. of calcium carbonate per acre.
N_2 = double the above amount of nitrogen.

The oats were planted on June 11, 1942, and germinated on June 17. On June 19 the plants were thinned to 35 plants per pot in all pots. On June 20 the first measurements of height were made and continued twice weekly at definite intervals until the experiment terminated on October 30, 1942. The pots all received a definite and equal amount of water at regular intervals throughout the experiment. The plants to be measured at any one time were chosen at random by measuring the heights of all the plants situated along a definite direction in the pot and on each occasion the direction was varied. Six to seven plants were measured each time and the mean value taken to give the height of the crop on that occasion. The heights were measured in centimetres.

The measurements were obtained over the whole period of 136 days, commencing with the time of germination as zero time. On examination of the curves obtained for the change of height with time it was found that, after the 60th to 70th day, variations became large and that the interpre a ion of the course of growth became difficult. The data became complicated by the effects of morphological changes in the plants due to factors such as flowering and setting of the seed. For these reasons it is not proposed to analyse the curves obtained beyond the 70th day. In some cases the changes are not followed further than about the 60th day, since the onset of flowering, etc., varied in time in the different pots. The effect of morphological changes upon the course of growth has been discussed by Briggs, Kidd and West[1] in the case of maize and it was shown that the time of incidence of such changes may be influenced by external factors and that changes occur in the rate of growth at these times.

On examination of the curves obtained in the present work showing the increase with time of the height of the oat plants during the period of the first 60–70 days it was found that three successive cycles of development were clearly shown. Such successive cycles in the growth of an organism are not uncommon in both animals and plants. Robertson[8] has dealt with this phenomenon in the case of human beings and developed the use of cycles "fixed" at their extremities in which each cycle is characterized by constants appropriate to that cycle. As a preliminary study of the course of changes in height, the constants for each of the three successive cycles were calculated according to equation (1) and it became apparent that the course of the first cycle of growth in all cases was almost identical and lasted for about two weeks before the second cycle became apparent. On this account no attempt was made to apply equation (2) to the data for this cycle in order to determine in detail any possible effects due to external and internal conditions separately. The similarity in the initial cycles of growth is shown by the constants reported in Table I and obtained from the data in accordance with equation (1). Logarithms to the base 10 were used, the values of "a"$_1$ are given in centimetres and the time "t_0" in days from the time of germination. "a_1" is the height attained during this first cycle.

TABLE I

	"a_1"	K	k	t_0
I	9·5	0·204	0·0257	5·5
II	9·5	0·248	0·0261	5·5
III	10·0	0·223	0·0223	6·0
IV	10·5	0·223	0·0212	6·0
V	9·8	0·260	0·0265	5·0
VI	10·0	0·234	0·0234	6·0
VII	10·5	0·240	0·0239	5·5
VIII	10·0	0·240	0·0240	6·0
Average ...	10·0	0·240	0·0240	6·0
Stand. dev. ...	±0·39	±0·0184	±0·00188	±0·5
Coeff. of var. (per cent)	3·9	7·7	7·8	8·3

It will be seen that the values for the constants "a_1", "k" and "t_0" are remarkably close in all cases, the coefficients of variation being 3·9, 7·8 and 8·3 per cent respectively. These results warrant the assumption that, during the first ten days of growth, the plant is not affected to any great extent by external factors such as treatment and that the nutritional level of the tissues in all cases is remarkably close. It would appear, therefore, that during this early cycle of growth the plant must depend mainly upon the reserves of plant food present in the seed at the time of planting. For this reason it has not been deemed necessary to attempt to analyse the data more carefully in an effort to distinguish between the effects due to external and internal conditions. The average values for the constants will represent the course of the growth in any one case with sufficient accuracy. Towards the end of this initial cycle of development a second cycle of growth commences and the effects of the different treatments become more obvious and the changes in each case cannot be represented by any single average curves for all the different treatments.

The increase of height of the oats which occurs during the second cycle of development, of course, will be added to the amount of growth which has already occurred during the first cycle so that if a_1 is the resultant height of the first cycle and x the increase of height from the beginning of the second cycle the total height of the plant will be $a_1 + x$. In the present case the value of 10·0 cm. was deducted from the recorded heights in order to obtain the values for the increase of height during the next cycle of growth. For comparison, the constants were calculated from the data for both equations (1) and (2). Using the same magnitudes as before, the constants for equation (1) are given in Table II; "a_2" is the increase in height, occurring during the second cycle.

These results have several points of interest. It is clear that the value of "a_2" varies with external conditions such as the different fertilizer treatments. The nutritional level of the tissues must be affected by such treatments and the value of "a_2" varies accordingly as suggested by Robertson. The results also indicate that K tends to remain more nearly constant and independent of environment than "k". Since $K = ka_2$, it is likely that the value of K represents the overall effect of factors which influence "a_2" and "k" separately and be expressive of the tendency of the organism to maintain the development as nearly normal as possible. The variations in the constant "k" do not support the contention that "k" is a specific inherent constant. The values indicate clearly that a high value of "k" is associated with a low value of "a_2" and that,

TABLE II

	a_2	K	k	t_0
I... ...	5·0	0·100	0·0200	23·0
II... ...	6·2	0·095	0·0153	22·5
III... ...	9·5	0·105	0·0111	26·0
IV... ...	9·0	0·142	0·0158	21·5
V... ...	13·5	0·112	0·0083	23·0
VI... ...	14·5	0·107	0·0074	23·5
VII... ...	14·0	0·102	0·0073	24·0
VIII... ...	16·0	0·102	0·0064	27·5
Mean ...	—	0·108	0·01145	—
Stand dev. ...	—	± 0·0146	± 0·00501	—
Coeff. of var. (per cent)	—	13·5	43·7	—

therefore, "k" is subject to variations according to external conditions. Since "k" varies inversely with "a_2" it is possible that the constant K might more nearly fulfil the requirements of a specific constant such as required by Robertson. The values for the coefficients of variation indicate such a tendency. It is interesting to note that the values of "t_0" vary slightly for the different treatments but that, for all the treatments, the period of maximum growth rate lies within a period of a week.

In view of the above conclusions it was felt that a more detailed analysis was necessary and therefore the constants for this second cycle of development were calculated in accordance with equation (2). Using the same magnitudes as before the results in Table III were obtained.

TABLE III

	a_2	$k_1 + k_2a_2$	k_1	k_2	t_0
I	5·0	0·0651	0·0302	0·00718	16·0
II	6·2	0·0656	0·0241	0·00670	16·5
III	9·5	0·0785	0·0116	0·00704	23·5
IV	9·0	0·0903	0·0278	0·00695	17·0
V	13·5	0·0945	0·0107	0·00621	21·0
VI	14·5	0·0933	0·0037	0·00618	23·0
VII	14·0	0·0922	0·0075	0·00625	22·5
VIII	16·0	0·1010	0·0025	0·00615	26·5
Mean	—	0·0851	0·0148	0·00658	—
Stand. dev. ...	—	± 0·0137	± 0·0110	± 0·000435	—
Coeff. of var. (per cent)	—	16·1	74·3	6·6	—

The height of the oats attained during this cycle of development must obviously be the resultant of all the growth factors in operation and must remain the same no matter what form of expression may be used to describe the changes that occur. There is thus no difference in the value of "a_2" for equations (1) and (2). The value of "$k_1 + k_2a_2$" was found, in general, to increase with the increase in final height as measured by "a", but no clear-cut conclusions can be drawn concerning this factor, which represents the combined effects of various factors. The value of the constant "k_1" clearly varies with the external conditions in an inverse ratio to the value of "a_2" the resultant height-yield of this second cycle of growth. It must be concluded that a lower value of "k_1" is associated with more favourable growth conditions. It is

interesting to note that a "complete" fertilizer treatment is more effective than any other treatment since the value of "k_1" is lowered appreciably by such treatment. In this respect "k_1" may be regarded as an "external" constant and should be of value in crop studies. However, the values of "k_2" are of special interest. The close agreement between the values of "k_2" for the various treatments is extremely significant. The variation in the value of this constant is remarkably small when compared with the variations in the value of "k_1", the mean value being 0·00658 with a coefficient of variation of only 6·6 per cent. There is clear evidence, therefore, that "k_2" has the properties of a specific inherent constant, independent of external conditions; "k_2" may be regarded as an "internal" constant and, as such, should have a special significance in varietal and racial studies in plants and animals. It must be remembered that in the present case the value of "k_2" may be influenced by the errors introduced into the first cycle, where an average curve was assumed to express the growth changes in all the treatments. It will be noticed that the value of t_0, the period of maximum growth rate, actually occurs earlier than suggested by the value of t_0 as given in Table II. At the same time this period in this case shows a wider variation for the different treatment. Since the value of t_0 is governed by the relative values of "k_1" and "k_2" it might be expected that differences in treatment would have some such effect.

The second cycle of growth covered a period of 3–4 weeks, after which a third cycle of development commenced and also lasted for 3–4 weeks. This cycle will, of course, be characterized by the increase of height which occurs after the completion of the second cycle. Thus, if "a_1" and "a_2" are the heights reached in each of the two previous cycles and x the increase of height since the commencement of the third cycle the total height of the plant will be given by a_1+a_2+x. The data for the third cycle, therefore, were obtained by deducting the values of $a_1 + a_2$ from the observed heights during this last cycle. The values of the constants for equations (1) and (2) were calculated in the same way as before and the constants for equation (1) are given in Table IV. "a_3" is the increase in height during the third cycle.

TABLE IV

	a_3	K	k	t_0
I	3·25	0·108	0·0322	54·0
II	6·1	0·122	0·0200	47·5
III	10·0	0·103	0·0103	48·0
IV	4·5	0·105	0·0233	51·0
V	10·8	0·143	0·0131	46·5
VI	10·5	0·154	0·0146	46·0
VII	14·5	0·172	0·0123	49·5
VIII	12·0	0·160	0·0133	50·0
Mean ...	—	0·133	0·0175	—
Stand. dev. ...	—	± 0·0256	± 0·00766	—
Coeff. of var. (per cent)	—	19·3	43·8	

Similar conclusions regarding the variations in the constants can be made in the case of the above results as were made in the case of the corresponding constants for the second cycle. The variations in the value of "k" are too large to support the view that "k" is a specific constant, independent of external conditions. It will be noetd that the value of "k" varies inversely with the value of "a_3".

The constants for the third cycle of development were also calculated in accordance with the modified autocatalytic equation (2) and the results are given in Table V.

TABLE V

	a_3	$-k_1+k_2a_3$	k_1	k_2	t_0
I	3·25	0·0612	0·0176	0·0136	49·0
II	6·1	0·0893	0·0070	0·0135	46·5
III	10·0	0·1212	0·0012	0·0120	47·5
IV	4·5	0·0685	0·0109	0·0128	49·0
V	10·8	0·1360	0·0030	0·0123	46·0
VI	10·5	0·1378	0·0065	0·0125	45·5
VII	14·5	0·1720	0·0010	0·0118	49·5
VIII	12·0	0·1483	0·0043	0·0120	49·5
Mean	—	0·1168	0·0064	0·0126	—
Stand. dev. ...	—	.. 0·0398	.. 0·00563	.. 0·0007	—
Coeff. of var. (per cent)	—	33·8	88·0	5·5	—

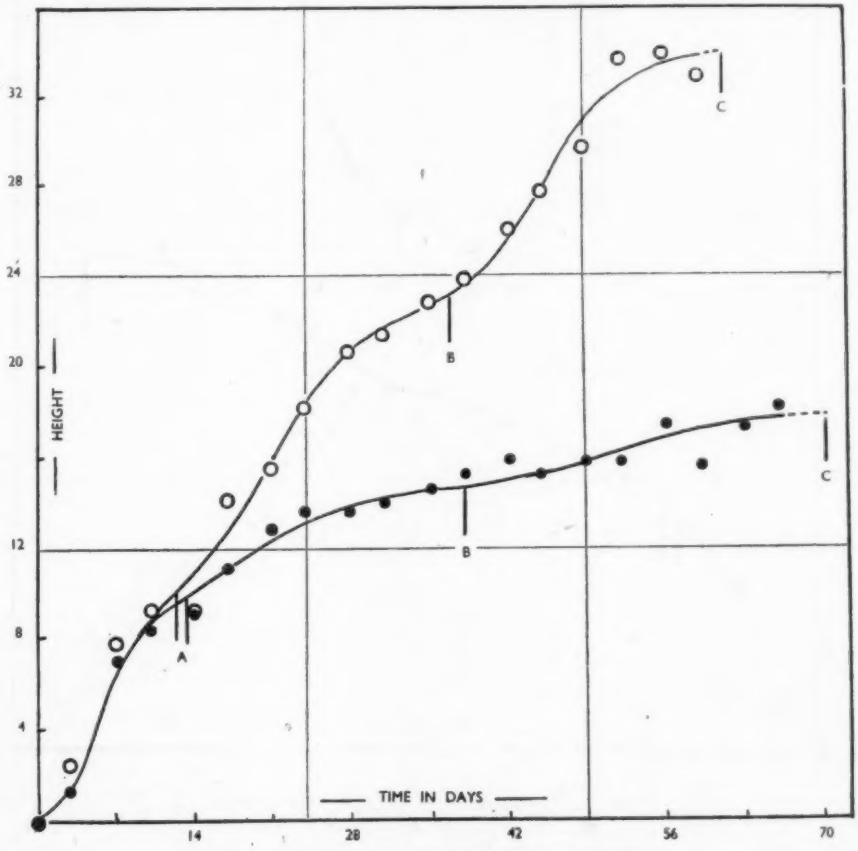

FIG. 1. INCREASE IN HEIGHT OF OATS
● = Treatment I O = Treatment V

The variations in the values of the constant show exactly similar tendencies as the constants for the second cycle of growth in Table III. It will be seen that the value of "k_1" shows comparatively large variations in accordance with variations in external conditions. The value of "k_2", however, remains practically constant, the average value being 0·0126 with a coefficient of variation of only 5·5 per cent. This value of "k_2" is very nearly double the value for the previous cycle and may be regarded as a specific inherent constant for this crop during this cycle of growth.

The agreement between the observed and calculated results for the three cycles of growth when equation (2) is applied to the data is shown in Figs. 1–4.

In Figs. 1–4, the portion of the curve from zero time to the point marked A represents

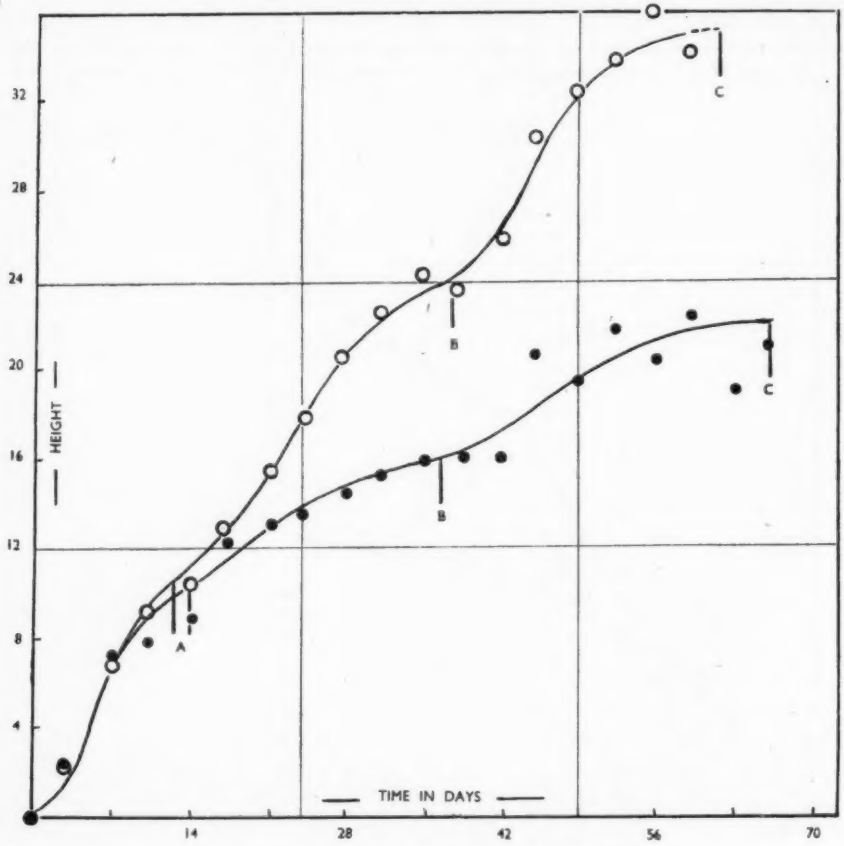

FIG. 2. INCREASE IN HEIGHT OF OATS
● = Treatment II O = Treatment VI

the first cycle of growth, the portion between this point and the point B represents the second cycle of growth. The third cycle is represented by the curve from the point B onwards. At the points A and B there is a fusion of two cycles of growth; but, as the one cycle flows into the next, there is no clear-cut separation. If a_1 represents the height attained during the first cycle and a_2 the increase in height during the second cycle, the total height at A will be a_1 and at B the height will be a_1+a_2. Similarly, at the end of the cycle at the point C, the height will be $a_1+a_2+a_3$, where a_3 is the increase height during the third cycle.

In order to compare the closeness of fit to the observed data in the case of equations (1) and (2), the deviation $\triangle$, the sum of the squared deviations $\triangle^2$, and the value of R for both sets are given in Table VI. "R" is calculated according to the method of Yule and Kendall.[9]

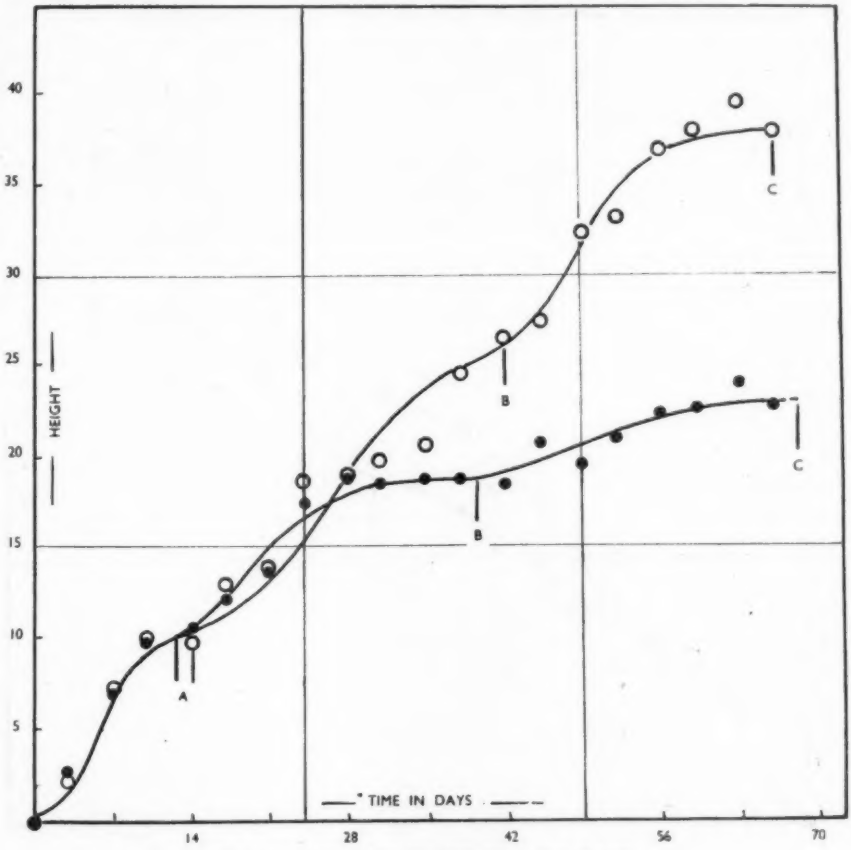

FIG. 3. INCREASE IN HEIGHT OF OATS
● = Treatment III O = Treatment VII

TABLE VI

	Equation I			*Equation II*		
	△	△²	R	△	△²	R
I	− 0·95	8·65	0·9910	− 1·25	7·83	0·9918
II	− 6·75	31·45	0·9805	− 4·15	25·98	0·9835
III	+ 1·25	18·65	0·9925	− 0·45	18·75	0·9927
IV	+ 1·40	10·94	0·9945	+ 0·80	11·90	0·9937
V	+ 1·70	14·36	0·9960	+ 0·15	12·48	0·9967
VI	− 0·30	6·42	0·9985	+ 0·75	5·45	0·9987
VII	+ 4·40	17·19	0·9961	+ 3·35	15·23	0·9966
VIII	+ 6·60	38·86	0·9934	+ 4·10	35·75	0·9939

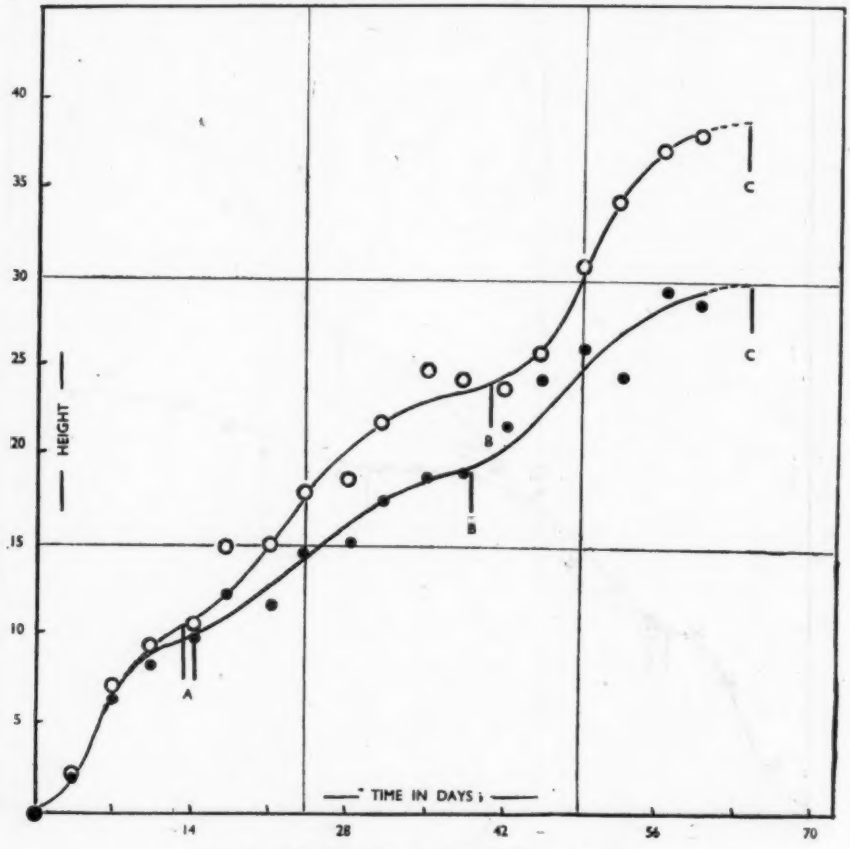

FIG. 4. INCREASE IN HEIGHT OF OATS
● = Treatment IV O = Treatment VIII

With the exception of treatments I and IV the deviations for the three cycles of growth are less for equation (2) but, in any case, the differences between the deviations in the two cases are not significant. In treatments III and IV the sums of the squared deviations for equation (2) are slightly higher than for equation (1) but the differences are insignificant. In all cases except in treatment IV the closeness of fit, measured by R, is better in the case of equation (2). It may be concluded that equation (2) provides a better description of the changes which occur during the development of the oat plant in terms of height. The constants "k_1" and "k_2" more nearly represent the effects of external and internal factors on the growth processes, and possess much greater significance than the simple constant "k" of equation (1). They should prove of great value in various studies of crops and animals.

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THE SAPONINS

PART II. THE ISOLATION OF GITOGENIN AND DIGITOGENIN FROM *CESTRUM PARQUI*

by

P. A. S. CANHAM and F. L. WARREN

OPSOMMING

Daar word aangetoon dat die bessies en blare van *Cestrum parqui*, saponiene bevat. Hydroliese van die gemengde saponiene lewer die sapogiiniene op. Laasgenoemde word as die asetate geskei en is as gitogenin en digitogenin geidentifiseer.

SUMMARY

The green berries and leaves of *Cestrum parqui* are shown to contain saponins. Hydrolysis of the mixed saponins gave the sapogenins which were separated as their acetates and identified as gitogenin and digitogenin.

In a recent publication[1] it was shown that *Cestrum laevigatum* Schlecht contained two saponins which were hydrolysed to gitogenin and digitogenin. It is of interest to note that two other species of the same genus have been reported as containing uncharacterized alkaloids. *C. foetidissimum* Jacq. is said to give a solanaceous alkaloid[2], whilst *C. parqui* L'Herit was found by Mercier and Chevalier[3] to have a toxic alkaloid, parquin, $C_{21}H_{39}O_8N(?)$, m.p. 180–181°, and a glycoside. This glycoside was hydrolysed by an enzyme in the plant to valeric acid and a new glycoside which yielded a phytosterol-like substance.

C. parqui, the willow-leaved jessamine, is indigenous to Chile, and berries and leaves of this plant were obtained from the poisonous plant garden of the Allerton Veterinary Research Laboratories, Natal, through the kindness of the Director, Dr. A. S. Canham.

Experiments done on the leaves and berries showed the presence of saponins, but we failed to isolate parquin, which is reported as unstable to light and air. Alcoholic extraction gave a glycosidal mixture which could not be crystallized or separated into pure components.

Hydrolysis of the glycosidal mixture gave reducing sugars and a solid having a melting point 283°, which remained constant under different conditions of crystallization. Acetylation, however, gave a mixture of acetates which was readily separated into gitogenin diacetate and digitogenin triacetate. Mixed melting points done with gitogenin diacetate and digitogenin triacetate respectively, obtained from *Cestrum laevigatum*[1] showed no depression. The two acetates were readily hydrolysed to digitogenin and gitogenin.

	Cestrum parqui	Cestrum laevigatum	*Recorded melting points*
Gitogenin	269–272°	268–270°[1]	272°, 268°[5]
Gitogenin diacetate	242°	242°[1]	242°[5]
Digitogenin	284–285°	284–285°[1]	284°[6]
Digitogenin triacetate	190°	190°	190°[6]

Thus both *C. laevigatum*[1] and *C. parqui* (*Solanaceae*, order *Tubiflorae*) contain a mixture of saponins which are hydrolysed to gitogenin and digitogenin, and which also occur together as glycosides in *Digitalis purpurea* (*Scrophulariaceae*, order *Tubiflorae*).

The toxicity of the leaves and fruit of *C. parqui* was determined by Descazeau[4] who reported that the course of the disease was rapid. This is in conformity with the rapid lethal effect of the same glycosidal mixture, obtained from *C. laevigatum*[1], and the toxicity of *C. parqui* is almost certainly due to the saponins and the symptoms similar to those of the "Chase Valley" disease of Natal.

EXPERIMENTAL

Extraction of saponins

Fresh green berries (150 g.) were minced up and extracted five times with boiling 95 per cent ethanol (5 x 250 ml.), the extract concentrated under reduced pressure until further distillation became impracticable owing to excessive foaming. The concentrate was washed with ether to remove fats and chlorophyll, when most of the mixed glycosides separated between the aqueous and ether layers. The mixture of glycosides, filtered off and washed well with ether, was obtained as a white amorphous solid (1·5 g.) The yield from the leaves was very much less.

Acid hydrolysis of the mixed glycoside

The mixed glycoside (500 mg.) was refluxed with a mixture of ethanol (20 ml.) and concentrated hydrochloric acid (5 ml.) and, after one hour, crystals began to separate. After three hours the solution was poured into water and extracted with ether. The ether was washed well with water and on evaporation deposited a mixture of aglycones as long needles, m.p. 283°, unchanged by repeated crystallization.

Separation and identification of gitogenin and digitogenin

The mixed aglycone (1 g.) was acetylated by boiling with acetic anhydride and worked up as previously described.[1] The product was crystallized twice from ethanol to give gitogenin diacetate, m.p. 242°.

Found: C, 72·4; H, 9·6.
$C_{31}H_{48}O_6$ requires: C, 72·1; H, 9·4%.

This acetate (1 g.) was hydrolysed with 10 per cent alcoholic potassium hydroxide (50 ml.). The crude solid was crystallized from ethanol to give long, colourless needles of gitogenin, m.p. 269–272°.

Found: C, 74·8; H, 10·3.
$C_{27}H_{44}O_4$ requires: C, 75·0; H, 10·3%.

The mother liquor from the crystallization of gitogenin diacetate on concentration gave digitogenin triacetate as long needles, m.p. 190°, which was unaltered by recrystallization.

Found: C, 68·9; H, 8·6.
$C_{33}H_{50}O_8$ requires: C, 69·0; H, 8·8%.

Hydrolysis of this acetate as described above and crystallization of the product from ethanol gave digitogenin as long needles, m.p. 284–285°.

Found: C, 72·3; H, 10·3.
$C_{27}H_{44}O_5$ requires: C, 72·3; H, 9·9%.

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NITRIC ACID OXIDATION OF COAL

PART I. IDENTIFICATION OF SOME COMPONENTS OF THE ETHER-SOLUBLE FRACTION

by

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OPSOMMING

'n Literatuuroorsig van steenkooloksidasie word gegee en nuwe eksperimente, waar salpetersuur gebruik word as oksideermiddel, word beskrywe. Distillasie, in hoogvakuum, van die eter-oplosbare gedeelte van die oksidasieproduk lewer barnsteensuur, ftaalsuur en hul ooreenkomstige anhidriedes en pikriensuur. Behalwe hierdie produkte word 'n mengsel van neutrale, kristallyne en vloeibare stowwe verkry.

SUMMARY

The literature on oxidation of coal is summarized and new experiments are described using nitric acid as the oxidizing agent. The ether-soluble fraction of the oxidation product yielded succinic acid and its anhydride, phthalic acid and its anhydride and picric acid on high-vacuum distillation. A mixture of neutral crystalline solids and mobile liquids was also obtained.

Of the various methods used for the investigation of the chemical constitution of coal, that of oxidative degradation has yielded the most promising results in view of the large number of organic compounds of medium molecular weight obtained from coal by this method of degradation.

In 1891, Friswill[1] prepared alkali-soluble substances by treatment of coal with dilute nitric acid. This publication formed the first of a long series on the oxidation products of coal. A large variety of products have been obtained:—

(*a*) Simple aliphatic acids: oxalic and acetic acids,[2] succinic acid,[4] propionic, butyric and caproic acids.[3]

(*b*) Benzene carboxylic acids: terephthalic acid, benzene tricarboxylic and benzene tetracarboxylic acids,[2] benzene pentacarboxylic acid and mellitic acid.[4]

(*c*) Picric acid.[2, 4]

(*d*) Coloured soluble acids: Various workers[5,6] have mentioned the presence of coloured water-soluble acids in the nitric acid oxidation product of coal. These acids, which vary in colour from light yellow to intense orange, are usually obtained as mixtures. The mixtures have not as yet been separated and the substances have not been obtained crystalline. Their esters have been prepared, but a large fraction of the esters do not distil in a high vacuum.[6] The thermal instability of these products cannot be accounted for solely by the presence of hydroxyl and carboxyl groups, since the esters are also unstable.[7]

According to Howard[7] these acids probably do not contain a single type of nucleus as in the benzene carboxylic acids in which an increase in molecular weight is caused entirely by addition of peripheral groups, but rather one in which an increase in molecular weight is caused both by increase in nuclear size and addition of peripheral groups.

These coloured acids are also obtained by the oxidation of coal with alkaline permanganate[6] as well as by air oxidation.[9]

Preliminary experiments on the chromatographic separation of the products have been conducted.[10]

The complexity of these acids is such that any conclusions drawn may be of significance in elucidating the chemical constitution of coal.

(*e*) Humic acids: These are obtained by the mild oxidation of coal with dilute

nitric acid.[11, 12, 13] They are considered to be hydroxy carboxylic acids. Molecular weights, equivalent weights and ultimate analyses indicate that the humic acids obtained by different methods of oxidation are closely similar. Although structural formulae have been proposed for these substances[14] very little is known of these acids, except that they are complex cyclic organic molecules as is suggested by carbon-hydrogen ratios obtained by ultimate analysis, and high light absorption. It has been suggested that *iso*-nitroso groupings account for the high percentage of nitrogen found in the so-called "nitrohumic acids.[15] These acids can be degraded further by nitric acid oxidation (as well as by other methods) to the intermediate soluble acids or to simple aliphatic- and benzene-carboxylic acids.[2] This suggests a stepwise oxidation, but there is evidence that all primary oxidation products do not yield the same products on further oxidation.

The initial reaction between coal and nitric acid (1: 1) is strongly exothermic and is accompanied by a copious evolution of nitrogen dioxide. The reaction proceeds in the cold for 24 hours, after which period further reaction takes place on heating under reflux. The colour of the solution does not become lighter on prolonged treatment with nitric acid and a dark brown insoluble residue is left even after boiling for 120 hours with concentrated nitric acid. The residue was removed by centrifuging, and discarded. The acid was removed by distillation under reduced pressure. Difficulty was encountered in drying the sticky residue and the possibility of removing water by azoetropic distillation with benzene or absolute alcohol was examined. The latter was most efficient and the product was obtained as a dry porous resinous solid which could be readily powdered.

Inorganic material, not only as iron and aluminium nitrates but also as metallic salts of the organic acids in the oxidation product, were found to be present in the oxidation product. This was removed by extraction of the material with methyl ethyl ketone, and acidification of the residue followed by extraction of the aqueous solution with the same solvent. Removal of the solvent left a brown hygroscopic product which was completely soluble in methyl ethyl ketone.

Preliminary attempts were made to separate the substances chromatographically. Various adsorbents were examined, viz., alumina, calcium sulphate and calcium carbonate. The best separation was obtained with alumina. The well-separated yellow bands were eluted with ethanol, ethanol-water, water and ammonia respectively. The yellow eluates, on evaporation to dryness in a vacuum, left orange or brown resinous products. Picric acid was the only crystalline substance isolated chromatographically.

The oxidation product, free from metals, was then separated into:—

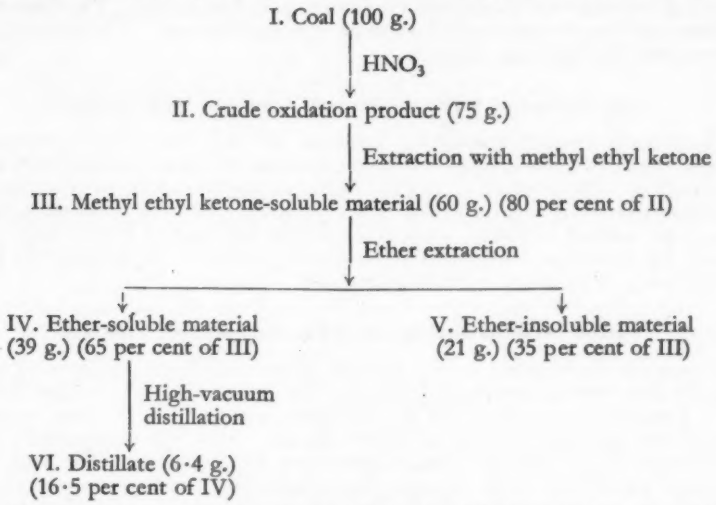
(*a*) An ether-soluble fraction which consisted of a reddish-brown viscous liquid which yielded distillable products in a high vacuum.

(*b*) An ether-insoluble fraction which was a dark-brown amorphous powder. This did not yield distillable products in a high vacuum. Investigations on this product are under way.

The ether-soluble fraction was distilled under high vacuum and the following sub stances isolated and identified: succinic acid, succinic anhydride, phthalic acid, phthalic anydride and picric acid.

A light-yellow, mobile liquid was also isolated. This liquid can also be obtained by direct extraction of the oxidation product from an alkaline solution with methyl ethyl ketone. The neutral product has been separated into a number of colourless and yellow liquids. The results are reserved for a later communication.

The yields of the various products described above are given in Table I below:—



From this table it is evident that only a small fraction (10·7 per cent) of the oxidation product (III) can be accounted for in the compounds isolated.

EXPERIMENTAL

Nitric acid oxidation of coal

Powdered coal (60 mesh, B.S.S., 125 g.) from Cornelia Colliery in the Vereeniging district (Transvaal) was treated with concentrated nitric acid (100 ml.) and allowed to stand for eight hours. Concentrated nitric acid (100 ml.) was added and allowed to stand for a further sixteen hours. The mixture was then boiled under reflux for two hours, by which time the evolution of brown fumes of nitrogen dioxide had ceased. The acid was evaporated until the volume of the solution was two-thirds the original volume; concentrated nitric acid (100 ml.) was added, the mixture boiled under reflux for ten hours and the acid concentrated again by evaporation of the dilute acid to two-thirds the original volume. This treatment was repeated six times so that the coal was heated with concentrated nitric acid under reflux for 62 hours. The dark-brown insoluble residue was separated from the opaque brown solution by centrifuging and treated with concentrated nitric acid for a further period of 60 hours as described above. The insoluble residue left after this treatment was again centrifuged, and discarded. The two nitric acid solutions were combined and the solvent removed by distillation under reduced pressure. The sticky resinous product was treated with absolute alcohol (100 ml.) and the solvent removed by distillation to leave a dark-brown resinous solid (93·7 g.).

Removal of inorganic material

The crude oxidation product (500 g.) was extracted with methyl ethyl ketone until the extract was almost colourless (2 l.). The residue was dissolved in 2N. hydrochloric

acid (750 ml.) the solution was saturated with sodium chloride and extracted with methyl ethyl ketone until the extract was almost colourless (3·5 l.). The solutions were combined and the solvents distilled off under reduced pressure. A brown hygroscopic resinous solid (400 g.) was obtained.

Separation into ether-soluble and ether-insoluble fractions

The oxidation product, freed from inorganic material (400 g.), was powdered and extracted with ether, using small volumes of the latter at a time, until no more material was extracted. The ethereal solution (3·25 l.) was filtered and the solvent distilled off. A reddish-brown viscous product (258 g.) was obtained. The residue of ether-insoluble material was washed with ether, powdered, and the last traces of ether-soluble material removed by extraction with ether in a Soxhlet apparatus for 48 hours. The product (139 g.) was a dark-brown amorphous powder.

High vacuum distillation of the ether-soluble material

The material was distilled from a specially constructed Pyrex distillation apparatus. The viscous material was heated and 25–35 g. allowed to run into the distillation flask. Degassed first at 97°C./10 cm. for fifteen minutes, then at room temperature and 0·05 mm. pressure for a further fifteen minutes. Heat was then carefully applied. Distillable products collected in the receiver from 75°C. upwards. The main fraction distilled at 130–170°C. The apparatus was rotated horizontally at regular intervals. At 230°C. no more material distilled over. The distillate (4·1 – 5·7 g.) collected in the receiver as a light-yellow oily product containing crystalline material.

Separation of distillate into solid and liquid fractions

Ether (100 ml.) was added to the distillate (50 g.) and the colourless crystalline material filtered off. The ethereal solution was allowed to stand for 24 hours when more material crystallized. This was filtered off and added to the rest of the solid material (total weight: 5·2 g.).

Separation of the solid distillate

The solid was washed with ether, pressed on a porous tile and the product sublimed at 55–60°C./0·05 mm. to give:—

(*a*) Colourless needles, m.p. 126–129°C. which collected in the cooler parts of the sublimation tube, and

(*b*) Colourless prisms, m.p. 116–119°C. solidified in the warmer parts of the tube.

These two fractions were resublimed:—

(*a*) Gave succinic anhydride (50 mg.) as colourless needles, m.p. 129–130°C., undepressed on mixing with an authentic specimen.

Found: C, 48·2; H, 4·2*
$C_4H_4O_3$ requires: C, 48·0; H, 4·0%.

(*b*) Gave phthalic anhydride (100 mg.) as prisms, m.p. 119–120°C., undepressed on mixing with an authentic specimen.

Found: C, 65·05; H, 2·9*
$C_8H_4O_3$ requires: C, 64·9; H, 2·7%.

*All micro-analyses, Drs. Weiler and Strauss, Oxford.

The residue in the sublimation tube was crystallized from a mixture of chloroform and alcohol and finally purified by vacuum sublimation to give succinic acid (2·5 g.), m.p. 187–188°C., undepressed on mixing with an authentic specimen.

Found: C, 41·2; H, 5·1*
$C_4H_6O_4$ requires: C, 40·7; H, 5·1%.
Equivalent weight: Found 58·2
$C_2H_4(COOH)_2$ requires: 59·0

Chromatographic separation of the liquid distillate

The oil (44·8 g.) was dissolved in dry ether (250 ml.), poured on a column of alumina, activated at 300°C. (equivalent to Brockman, grade II (1) ref. (16)). The column was developed with ether (300 ml.) and the various fractions eluted as indicated in the accompanying table.

Fraction	*Colour of band*	*Eluent*	*Volume of eluent*	*Nature of eluent*	*Weight (g.)*
I	Yellow	Ether	1000	Yellow oil and colourless solid	27
II	Yellow	Ethanol	600	Yellow needles and oil ...	1·7
III	Orange	2N. Ammonia	500	Brown resinous product (see below)	15

Fraction I (most weakly adsorbed fraction): The colourless crystalline solid was freed from the oil by washing with ether and crystallized from a mixture of acetone and chloroform to yield succinic acid (0·3 g.), m.p. 187–188°C. The ether washing gave an oil which is being investigated further.

Fraction II: The yellow crystalline substance was washed with ether to remove the oil. It was then crystallized from a mixture of chloroform and acetone to give yellow needles of sodium picrate (0·75 g.), m.p. 271–272°C. (decomposition) unchanged on further crystallization. The solid was dissolved in water and acidified with hydrochloric acid. The ether extract gave picric acid, m.p. 119–120°C., undepressed on mixing with an authentic specimen.

Fraction III: The ammoniacal solution was acidified and extracted with methyl ethyl ketone. The solvent was removed under reduced pressure and the resinous residue distilled at 80–120°C./0·05 mm. to give oily colourless needles and a carbonaceous residue. The crystals were pressed on a porous tile and resublimed at 74°C./0·05 mm. to give phthalic anhydride (0·2 g.) m.p. 119–120°C. The residue from the vacuum sublimation was crystallized from an alcohol-water mixture to yield phthalic acid (2 g.), m.p. 186–190°C. (in a sealed tube) undepressed on mixing with an authentic specimen.

Equivalent weight: Found: 85
$C_6H_4(COOH)_2$ requires : 83.

This investigation was conducted at the University of Natal and publication has been authorized by the Fuel Research Board.

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SENECIO ALKALOIDS

THE ULTRA-VIOLET EXTINCTION CURVES OF THE ALKALOIDS AND THE "NECIC" ACIDS

by

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OPSOMMING

Die ultraviolet-uitdowingskrommes van sommige van die „nesiese" sure en Senecioalkaloiede word verstrek en hulle belangrikheid in die struktuur van die alkaloiede word aangetoon. Retrorsien is uit *S. ruderalis* afgeskei.

SUMMARY

The ultra-violet extinction curves of some of the "necic" acids and *Senecio* alkaloids are recorded and their significance in the structure of the alkaloids is indicated. Retrorsine is isolated from *S. ruderalis*.

During the work on the "necic" acids[5, 6, 7, 8] the extinction curves in the ultra-violet were used to confirm the structures as $\alpha\beta$-unsaturated acids. It was observed[8] that whereas the wave-length of maximum absorption, λ_{max} was the same for all the "necic" acids, the molecular extinction coefficient (ϵ) at the maximum absorption (ϵ_{max} c.f. Braude[11]) varied with the geometrical configuration.

The orientation of isatinecic acid in retrorsine,[9] with which β-longilobine is identical,[10] and in isatidine[9] has been established in these laboratories. The identity of integerrinecic acid with *trans*-senecic acid,[8] makes integerrimine[1] (squalidine[8]) the only *Senecio* alkaloid known to have the acid in the *trans*-form. It was thought that the pronounced differences in ϵ_{max} might be used to determine the configurations of the acids in the alkaloids. This seemed important in view of the ready *cis-trans* isomerization of the acids under different conditions of hydrolysis.[5, 6, 8]

The extinction curves of the "necic" acids and of their parent alkaloids are shown in the figure. The molecular extinction coefficient was determined at intervals of 10–25A and the accuracy was estimated to be of the order of a quarter of one per cent. All points lay smoothly on the curves, which were reproducible: retrorsine from three different sources (i.e., from *S. isatideus* and from *S. retrorsus* as well as from isatidine by reduction) showed the same extinction curve (B^2).

The pronounced difference in ϵ_{max} for the *cis*- (curves A_3 and A_4) and *trans*- (curves A_1 and A_2) forms of the acids is very marked.

Substance	*Curve*	*Configuration*	λ *max.*	ϵ *max.*
Senecic acid	A_4	*cis-*	2185	4950
Isatinecic acid	A_3	*cis-*	2185	5450
Integerrinecic acid	A_2	*trans-*	2180	9250
Retronecic acid	A_1	*trans-*	2185	9350
Rosmarinine	B_4''	*cis-*	2180	6100
Isatidine	B_3	*cis-*	2175	6300
Platyphylline	B_4'	*cis-*	2190	6350
Retrorsine	B_2	*cis-*	2175	7100
Integerrimine	B_1	*trans-*	2160	8000

All the alkaloids show the maximum absorption at the same wave-lengths, which is that found for all the acids. Thus the same chromophoric grouping is present in the alkaloid as in the acid; and hydrolysis does not effect structural changes other than possible geometrical isomerism previously established.

Furthermore, the pronounced difference in ϵ_{max} for the *cis*- and *trans*- forms of the acids is not revealed for the alkaloids, the extinction curves for which lie intermediate between those of the two geometrical extremes. On the other hand, integerrimine, the only alkaloid containing the acid combined in the *trans*-form, shows the highest value for ϵ_{max}. The curves for platyphylline and rosmarinine (hydroxyplatyphylline) show close similarity, as would be expected from structural considerations. Finally, it is of interest that the three alkaloids (IIa, IIb and IIe) containing retronecine or its N-oxide reveal the presence of a maximum in the immediate far ultra-violet due to the ethylenic linkage (curves B_2, B_3 and B_1), an observation which may be used for structural determinations. The identity of retrorsine from *S. ruderalis* was confirmed by the extinction curve.

EXPERIMENTAL

The alkaloids and acids were prepared as below and crystallized to constant melting-point as recorded in the papers referred to below.

Retrorsine (*a*) from *S. isatideus*;[4,2]

(*b*) kindly supplied in 1942 by Prof. H. de Waal[2];

(*c*) from the reduction of isatidine.[5]

Isatidine from *S. isatideus*.[2]
Platyphylline from *S. brachypodus*.[3]
Rosmarinine from *S. hygrophylus*.[3]
Senecic acid from the hydrolysis of rosmarinine.[3]
Integerrinecic acid from the isomerization of senecic acid.[3]
Isatinecic acid from the hydrolysis of isatidine.[6]
Retronecic acid from the hydrolysis of isatidine and retrorsine.[6]

The extinction curves were determined in distilled water on a Beckmann Model DU Quartz Spectrophotometer.

Extraction of S. ruderalis: The alkaloid, extracted by the improved procedure of Koekemoer and Warren,[12] was crystallized from acetone to give retrorsine, m.p. 204–205° $[\alpha]_D^{20°}$–55°.

Found: C, 61·65; H, 7·2
$C_{18}H_{25}O_6N$ requires: C, 61·6; H, 7·2%.

The melting-point was undepressed on admixture with an authentic specimen. This gave an extinction curve identical with curve B_2.

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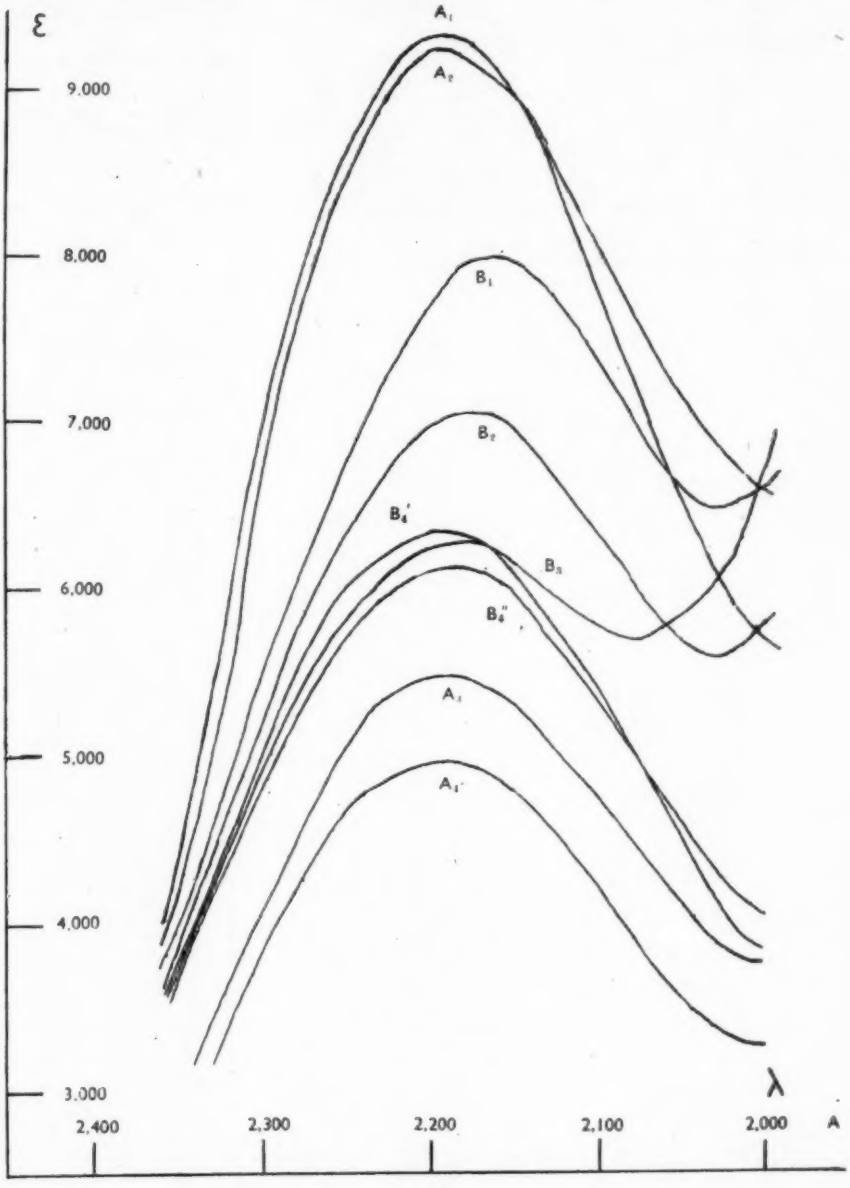
ε
9.000
8.000
7.000
6.000
5.000
4.000
3.000
A1
A2
B1
B2
B4'
B3
B4''
A3
A4
λ
2,400
2,300
2,200
2,100
2,000
A

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